THE STRIATO-NIGRAL PATHWAY:

Its role in the regulation of dopamine metabolism.

By

Marianela Garcia-Muñoz.

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The brain is wider than the sky
for put them side by side
the one the other will contain
with ease you and beside.

Emily Dickinson (1830-1886).

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The work presented in this thesis has been composed by myself with the following exceptions:-

A. The estimations of HVA, DOPAC, and GABA concentrations, described in Chapter II, sections C.3.3 and C.3.4 were performed by Mr. N. Nicolau.

B. The estimations of Dopamine concentrations, described in Chapter II, section C.3.2 were performed by Mrs. A. Wright.

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Marianela Garcia-Muñoz, M.Sc.

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ABSTRACT.

In rats, unilateral lesions in the ventro-medial area of the crus cerebri, where the striato-nigral pathway runs, produced a significant decrease in the concentration of substance-P, Gamma-amino-butyric-acid and glutamic-acid-decarboxylase activity in the substantia nigra ipsilateral to the lesioned side. The striato-nigral pathway has been proposed to mediate the effects produced by the systemic administration of dopaminergic agonists, (e.g. apomorphine or amphetamine) and antagonists (e.g. haloperidol) on dopamine metabolism in nigral cells. However, the lesions reported here do not alter the concentration of dopamine or its metabolites homovallinic-acid and dihydroxyphenylacetic acid in the striatum, in comparison with control animals, even after the administration of haloperidol and apomorphine. Recordings of dopaminergic nigral cells in rats with chronic and acute electrolytic lesions in the crus cerebri or with kainic acid lesions in the striatum, did not differ from recordings done in control animals. An intravenous administration of amphetamine, significantly reduced the firing rate of dopamine nigral cells in both control and lesioned animals. Similarly, haloperidol blocked the effect of a subsequent administration of amphetamine in both groups. The only lesions which effectively abolished the inhibition of nigral cell activity after amphetamine injections were those which destroyed some of the dopamine cells. These results are discussed as evidence for an alternative mechanism responsible for the observed inhibition which could also be involved in the regulation of dopamine metabolism. A behavioral study shows that lesions in the striato-nigral pathway induced after apomorphine a dose-dependent turning towards the lesioned side and significantly/

significantly decreased the number of turns induced in a 6-hydroxydopamine lesioned animal after apomorphine. Rats also changed their paw preference during lever pressing after a crus cerebri lesion contralateral to the prefered paw. A possible function for the striato-nigral pathway in the expression of motor behaviour is discussed.

GENERAL INTRODUCTION.

In 1963, Arvid Carlsson (48), while investigating the effects of antipsychotic drugs, (neuroleptics) on dopamine turnover in the striatum, proposed a feedback loop (substantia nigra-striatum-substantia nigra) suggesting an important role for the striato-nigral pathway in the control of dopamine metabolism. It was proposed that by means of this loop, dopaminergic transmission in the striatum results in decreased activity in dopaminergic neurons, while blockade of dopaminergic transmission in the striatum produces increased activity in dopaminergic neurons in the substantia nigra. Since this hypothesis was proposed, evidence in its favour has accumulated. A large number of reports using different techniques suggest that antipsychotic drugs and dopaminergic agonists exert opposite effects on dopamine turnover, (238, 11, 61, 218). Further support for this hypothesis came from evidence that systemic administration of dopaminergic agonists produces a marked inhibition of neuronal firing of dopamine cells in the substantia nigra while antipsychotic drugs reversed the effect of dopaminergic agonists, (2, 41, 1). This coincided with the postulation that amphetamine does not change dopamine metabolism because the feedback compensated the drug induced release, (48). A finding that fitted into this idea of feedback regulation was that gamma-amino-butyric-acid (GABA), postulated as an inhibitory transmitter in the nervous system, (252), was found in the substantia nigra, (226). Even more, GABA was decreased in this area after a hemisection between the substantia nigra and the striatum, (200) or striatal lesion, (278). This was taken as evidence to support the idea that antipsychotic drugs inhibited GABA containing neurons in the striatum which send their axons to the substantia/

substantia nigra. Antipsychotic drugs were postulated to decrease the amounts of GABA in the substantia nigra, disinhibiting dopamine neurons and in turn increasing dopamine synthesis, (135). An anatomical and biochemical analysis of the distribution of the activity of the synthetic enzyme of GABA. glutamic acid decarboxylase, (GAD) in the substantia nigra of cats, indicated that the loss of enzyme activity was localized and related to the site of termination of the degenerating striato-nigral fibres, (111). These results left the striato-nigral pathway as a GABA containing one and the most likely for the proposed feedback.

However, not all the evidence has been in favour of the hypothesis of feedback regulation. For instance, iontophoretic administration of dopamine into substantia nigral cells, reduce their firing rate (39,41) as it does in the striatum, (102, 303), suggesting that substantia nigral cells are also responding to their own transmitter. Also, release of dopamine within the substantia nigra has been observed in studies using in vitro and in vivo methods, (32, 217). This has suggested the possibility of alternative mechanisms to explain the effect of dopamine agonists and antipsychotic drugs in the metabolism of dopamine. In particular, it has been proposed that the blockade of dopamine receptors in the substantia nigra could relieve dopamine neurons from the inhibitory action of dopamine released within the substantia nigra, possibly by dendrites or axon collaterals of the same dopamine cells, (119).

A way of testing whether this local control of dopamine neurons is in fact operating or if the metabolism of dopamine is rather mediated by striatal neurons, is to study the effect of dopaminergic agonists and/

and antipsychotic drugs on animals whose main striatal influence is interrupted. So far, attempts to lesion the striato-nigral pathway (200, 40, 130) are open to criticism because the lesion has not been localized. Since the nigro-striatal and the striato-nigral pathways run close together, if the lesion is not small and circumscribed there is a high probability of damage to the nigro-striatal fibres and even of nigral cells. Based on the anatomical tracing studies of the striato-nigral pathway done in our laboratory by Tulloch et al (282), evidence is presented of lesions of the striato-nigral pathway which do not affect the nigro-striatal bundle. With these lesions, it was possible to study the effects of drugs which alter dopamine metabolism, on animals which did not have the possible control exerted by the striatum on dopamine cells proposed by the feedback hypothesis. The results obtained with this technique point out that the idea of a feedback loop has resulted in an oversimplification and that alternative mechanisms for the control of dopamine metabolism must be considered.

The first Chapter gives a critical review of the most relevant experiments in favour of or against the feedback hypothesis. The rest of the work presents biochemical, electrophysiological and behavioural evidence that suggests that the idea of a feedback loop is not sufficient to explain the control of dopamine metabolism. Other possible mechanisms to explain this control are discussed, as well as the possible function for a pathway which can no longer be considered as only part of a feedback system.

CHAPTER I

THE FEEDBACK PATHWAY

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THE FEEDBACK PATHWAY.

It is now 15 years since Carlsson and Lindqvist, (48) postulated that if chlorpromazine and haloperidol block monoaminergic receptors then: "... it does not seem unreasonable to assume that this receptor blockade results in compensatory activation of monoaminergic neurons. This activation 'leading to increased release of monoamines and consequently to increased formation of monoamine metabolites...", (pag. 141).

Since then, many investigators using different techniques, have obtained evidence in favour of or against this hypothesis. For instance, anatomical results confirm that there are fibres interconnecting the nuclei. The feedback hypothesis has its origins in biochemistry, and strong evidence in its favour has been obtained from this area, although there are also experiments which give evidence against it. Several alternative explanations to the feedback hypothesis have been proposed, but none of them at the moment is completely satisfactory. Electrophysiological experiments have demonstrated the effect of several drugs on the activity of the cells in the substantia nigra and the striatum, as well as their response to the stimulation of the other nucleus. Not all the results obtained in this area agree with the feedback hypothesis and some results support the alternative models proposed by the biochemists. If the results obtained by anatomical, biochemical and electrophysiological techniques are regarded as a whole, the picture is confused. Not all the evidence agrees with the hypothesis and furthermore the evidence against it although/

although strong enough to put the feedback idea in doubt, does not give an alternative interpretation. In this Chapter an effort is made to examine most of these results, as well as the alternative models.

1. ANATOMICAL EVIDENCE.

One of the strongest evidence in favour of the feedback pathway has been the demonstration of the connections from the striatum to the substantia nigra, and from the substantia nigra to the striatum, without which the proposed hypothesis could not have been substantiated.

One of the first descriptions of the human substantia nigra was made by Vicq d'Azyr in 1786, (291) and it was described later in the rat by Bauer, (21) among others. The substantia nigra derives its name from the melanin pigment in its cells, which gives a dark colour to the nucleus in unstained sections. Melanin, however, is not present in some animals like the laboratory albino rat, (77). This nucleus lies in the mesencephalon above the cerebral peduncle on each side. It is classically divided in three areas: lateral, compacta and reticulata, (77). The lateral area is considered the oldest phylogenetically and is the smallest portion of the region in man. The pars compacta contains more numerous cells distributed close together, this is the area which contains dopaminergic cell bodies. In the pars reticulata the cells are sparse and this part of the substantia nigra, like the pallidum and the red nucleus contains iron, particularly in glial cells, (36). In rats, cells in the substantia nigra do not seem to have a particular shape and in general measure from 11-74 μm; in the pars compacta cells of 19 μm predominate, (156,136).

1A. THE STRIATO-NIGRAL AND PALLIDO-NIGRAL PATHWAYS.

It seems that in humans, monkeys, cats and rats, the biggest afferent connection to substantia nigra comes from the striatum, (276, 272, 273, 274, 259, 293, 168). Fibres leave the striatum in the internal capsule, travel through the globus pallidum giving a great number of fibres to this nucleus, and continue in the internal capsule until they enter the substantia nigra pars reticulata, by traversing the cerebral peduncle in a comb bundle, (293, 259).

In primates and cats, (not in the rat), it is possible to divide the striatum into caudate nucleus and putamen. Efferents from these regions seem to project with a topographical organization, although the details of its distribution are sometimes contradictory, (43, 273). Szabo, (276, 275, 273, 274, 277) using anterograde degeneration techniques, reported that the lateral areas of the caudate nucleus project mainly to the external segment of the globus pallidum, and the most medial caudate region mainly to the internal segment (cat's entopeduncular nucleus). The caudato nigral fibres end in the substantia nigra reticulata with a similar medio-lateral, and anterio-posterior distribution pattern. The tail of the caudate nucleus seems to send fibres to the lateral area of the substantia nigra reticulata. The anterior putamen appears to project almost exclusively to the globus pallidus, while the rest projects to both the globus pallidus and the posterior substantia nigra reticulata.

The evidence supporting the existence of pallido-nigral fibres is conflicting, (247, 154) probably because lesions in the globus pallidus damage afferent fibres from substantia nigra, which could confuse/

confuse the results. This connection was denied for sometime (247, 290) but was later confirmed in cats and monkeys (154, 212) using different staining techniques. Lesions in the medial globus pallidus in monkeys or in the intopeduncular nucleus in cats showed degeneration in the substantia nigra pars compacta (212).

The techniques using axonal degeneration give contradictory results probably because the localization and size of the lesions change. These techniques have some inherent problems, which make interpretation of the results even more uncertain; for example, the unspecific damage produced by the lesions, making it difficult to interprete which is anterograde, which retrograde and which is trans-synaptic, degeneration.

Many neuronal constituents synthesized in the cell body are transported down the axon to reach the terminals, (230, 182). Also proteins can be taken up by terminals and subsequently transported to the cell body, (179, 178). These two processes of anterograde and retrograde transport have been used recently to trace afferent and efferent pathways in the brain, (124, 298).

An autoradiographic method takes advantage of the anterograde transport. When a labeled protein precursor, like the tritiated amino-acid leucine, is injected directly into a nucleus it is taken up by the cell bodies at the site of the injection (but not by the axons that have cell bodies elsewhere), and is transported to the terminals which can then be identified by means of the effect of the emitted radiation on photographic emulsions, (72).

The retrograde transport of proteins is used to trace afferent pathways in the nervous system, (185, 213) by injecting mainly horseradish/

horseradish peroxidase and looking afterwards for a stained reaction product of the peroxidase in the cells.

Using these techniques efferent fibres from the striatum and afferents to substantia nigra have been traced. In cats and rats (128, 140, 43) it has been observed that the striatum sends fibres through the internal capsule to the globus pallidus and substantia nigra. The globus pallidus, in turn, sends fibres to the substantia nigra, although there is disagreement about the area (compacta or reticulata) that this nucleus innervates, (43, 140).

The topographical projection of the striatum to substantia nigra has been described in the rat using the retrograde transport of horseradish peroxidase (43) reporting similar results to the ones Szabo (273) described previously, although they do not agree about the antero-posterior arrangement of the efferents, from the tail of the striatum, even though they agree that these efferents project mainly to the lateral substantia nigra.

In our laboratory, Tulloch (282) using anterograde and retrograde transport techniques, confirmed that the striatum and globus pallidus project to substantia nigra reticulata. In agreement with previous reports (140, 212), it was found that the globus pallidum projections include the substantia nigra pars compacta. The tail of the striatum was found to send fibres mainly to substantia nigra pars lateralis.

Previously it was suggested that in the cat only 5% of caudate cells send their axons to distal places, (167), but in the rat using the horseradish peroxidase method 30-50% of the cells were labelled, all of them being medium size cells (12-20um), (43, 128, 167).

One/

One of the problems of the horseradish peroxidase technique is that it is uncertain whether or not damaged axons take up and transport proteins, retrogradely (213, 301). If they do, then one has to be very cautious about interpreting the results and probably only take into account those that prove to be consistent by various techniques.

1B. THE NIGRO-STRIATAL PATHWAY.

Attempts to trace nigro-striatal fibres by anterograde degeneration were controversial in the early reports although they have been consistently seen using retrograde degeneration methods, (99, 22). With the use of silver impregnation techniques that stain fine fibres and nerve terminals, it was possible to observe degeneration in monkeys, cats and rats in the striatum after substantia nigra compacta lesions, (55, 143, 209).

Formaldehyde condenses with the monoamines to form fluorophores which can be observed in the cells by fluorescence microscopy. The Falck and Hillarp technique (96) based on this principle has a high degree of sensitivity because it is based on a fluorescence reaction and not on a conventional staining procedure.

Using this technique, it was demonstrated that there are dopamine containing neurons in the substantia nigra compacta which send their axons to the striatum (4).

As expected, injections of horseradish peroxidase into the striatum labeled presumed dopamine-containing neurons in the substantia nigra compacta, (179).

Using different anatomical techniques, it is consistently found that the substantia nigra projects in a topographical arrangement to the/

the striatum, in monkeys, (285, 57, 55). Posterior and lateral areas of the substantia nigra project to posterior and lateral areas of the striatum; there is a disagreement as to whether the antero-medial substantia nigra projects to most of the striatum, (285) or only to the anterior areas, (55, 22).

From earlier studies it was thought that the fibres leaving the substantia nigra compacta joined the internal capsule to ascend to the striatum, (55, 4), however it was later demonstrated that first the axons accumulate medial to the substantia nigra coinciding with the medial forebrain bundle in the lateral part of the hypothalamus, before entering the internal capsule, (232, 22). In order to arrive at the striatum the fibres cross the globus pallidum, making it difficult to corroborate the existance of a nigro-pallidal projection, (22).

1C. PREVAILING INTERPRETATIONS.

These two pathways reciprocally connecting the striatum and the substantia nigra, give a basis for the idea of a "compensatory feedback mechanism". The turnover of dopamine according to this idea, depends on the activity of dopaminergic cells in the substantia nigra which are controlled by afferent fibres coming from the striatum. However, against this idea of feedback it can be argued that if dopamine containing cells projecting to the striatum are confined in the substantia nigra pars compacta, how is it that fibres coming from the striatum, ending in substantia nigra pars reticulata, exert a control over substantia nigra pars compacta cells? To keep the system connected, alternative explanations may be brought into play. Interneurons have been observed in substantia nigra, (264), which could close the feedback loop./

loop. Also numerous dendrites from cells in the pars compacta invade the zona reticulata, (264, 73) and axo-dendritic synapses could complete the system. There is also the possibility that fibres from the tail of the striatum or from globus pallidum, if ending in substantia nigra pars compacta, (140, 212, 282). could be involved in the mechanism of feedback control.

In general, the anatomy supports the idea of a reciprocal relation between substantia nigra and the striatum which may be considered as the basis for a feedback mechanism.

2. PHARMACOLOGICAL AND BIOCHEMICAL EVIDENCE.

The whole idea of a "compensatory feedback mechanism" was proposed to explain the effects of dopaminergic receptor blocking agents like haloperidol, chlorpromazine and other antipsychotic drugs, (neuroleptics).

These drugs have been shown to enhance the dopamine turnover in the striatum (Section 2A), and as postulated, block dopamine receptors and influence a negative feedback mechanism leading to activation of dopaminergic neurons, (48).

2A. EVIDENCE IN FAVOUR

A1. EFFECT OF DOPAMINE RECEPTOR AGONIST OR ANTAGONIST ON DOPAMINE TURNOVER.

A large number of reports suggest that the antipsychotic drugs increase dopamine turnover and to a lesser extent the turnover of norepinephrine,/

norepinephrine, (223, 225). Different catecholamine metabolites, like normetanephrine, 3-methoxytyramine (48), and 4-hydroxy-3-methoxy-phenylacetic acid, (Homovallinic acid, HVA), (238) and dihydroxyphenylacetic acid (Dopac), (254), are found to be increased after antipsychotic drugs.

If the synthesis of dopamine is blocked by inhibiting the enzyme that converts tyrosine into Dopa, tyrosine hydroxylase, with alpha methyl-para-tyrosine, (AMPT), the concentration of dopamine in the striatum is reduced, (69). By giving antipsychotics like haloperidol or chlorpromazine (11) the rate of disappearance of dopamine is increased, which also indicates that dopamine synthesis is enhanced after these drugs.

Apomorphine, a dopaminergic agonist (6, 93), causes opposite effects to those produced by antipsychotic drugs. For example, it caused a significant decrease in HVA in the striatum, (253); after AMPT, apomorphine decreases the rate of dopamine disappearance (11). After inhibition of Dopa decarboxylase (the enzyme that converts Dopa into dopamine), the accumulation of Dopa is markedly inhibited, by apomorphine, (166).

The fact that the blockade or stimulation of dopamine receptors have opposite effects led to the conclusion that the feedback originates from the postsynaptic dopamine receptor activity.

Another method used to detect changes in catecholamine synthesis is the measuring of the accumulation of labelled dopamine formed from radioactive tyrosine (L-3,5-^{3}H tyrosine, (61) or ^{14}C tyrosine, (225), or the accumulation of tritiated water formed during the conversion of tritiated tyrosine into Dopa (123).

The/

The labelled tyrosine has been administered intravenously (61), by local microinjection (61) or by local superfusion from a cup placed at the surface of the caudate nucleus (118) or from a push-pull cannula inserted into the nucleus, (218). The way in which it is administered also changes the way in which the radioactive product is collected. However, despite the possible differences, advantages or disadvantages of the methods, the results are consistent. After antipsychotic drugs, the dopamine synthesis is increased (61, 123, 225, 223, 218).

A2. IMPULSE FLOW AND DOPAMINE TURNOVER.

The effect of chlor promazine on the accumulation and disappearance of dopamine formed from ^{14}C-tyrosine in different rat brain regions, (221, 222), indicated that the effect was confined to the striatum. Therefore it was thought, in accordance with the feedback hypothesis, that the effect of antipsychotic drugs may be induced by accelerating nerve impulse activity in the nigro-striatal pathway.

In support of this idea, it was shown that chlor promazine had no significant effect after the interruption of the nigro-striatal dopamine pathway, (225) and the disappearance of dopamine after AMPT was counteracted by a nigral lesion, (8). Thus suggesting that impulse flow was necessary for the antipsychotic drug induced increase in dopamine turnover.

Further evidence that gave support to the idea that increased impulse flow in dopamine cells was responsible for the increase in dopamine synthesis after dopamine receptor blockade came with the experiments on gamma-aminobutyric acid, (GABA) a known inhibitory transmitter (252) which was found in one of the highest concentrations

in/

in the substantia nigra (226, 278). Hemisection (200) or lesions in the striatum (169, 200) produced a marked decrease in GABA and its synthetic enzyme, glutamate decarboxylase (GAD, (278, 279). This suggested that antipsychotic drugs blocked dopamine receptors on GABA containing neurons in the striatum producing a decrease in GABA in the substantia nigra, with a consequent release of the dopamine cells from inhibition resulting in an increased impulse flow and dopamine synthesis. In support of this idea, it was shown that Muscimol, (GABA agonist) injected directly into substantia nigra concommitantly with a systemic injection of haloperidol reduced the haloperidol induced activation of TOH in the striatum by 50%, (135). According to this, antipsychotic drugs activate dopamine neurons and increase striatal TOH by causing a reduction in the release of GABA (as inhibitory transmitter) from the terminals of the striato-nigral pathway, (135). However, it has been shown that haloperidol produces an increase in the concentration of GABA (after GABA transaminase inhibition) in substantia nigra, (58). Therefore if GABA is considered as an inhibitory transmitter over dopamine cell bodies, then the increase in GABA accumulation after haloperidol cannot be directly correlated with the increase in dopamine turnover as a result of an increase in impulse flow.

2B. EVIDENCE AGAINST.

B1. IN VITRO STUDIES.

When the effects of systemic or intracerebral administration of drugs are estimated using intact brains, all sorts of interactions between structures can be proposed. The problem begins when these relations/

relations are maintained after sectioning the brain.

In vitro treatment of the rat striatal slices with neuroleptics, increased labelled dopamine over flow in response to field stimulation, (98, 97). Therefore the effects induced in vivo by different processes, can still be detected in vitro when the feedback cannot operate anymore.

While it cannot be excluded with this evidence that the feedback does exert any effect, it seems at least as likely that another kind of mechanism is also involved in the regulation of dopamine metabolism.

B2. PRESYNAPTIC RECEPTORS.

As was previously mentioned (section A2) there is evidence that nerve impulse flow is correlated with the synthesis of dopamine and the effects of antipsychotic drugs, (8). However, when the nigro-striatal fibres are interrupted, the synthesis in the terminal fibre system is not inhibited, but instead is stimulated, (166).

Interruption of impulse flow by electrothermic lesions, mechanical transection, or injection of local anaesthetics, (166, 164, 296), produces a marked increase in dopamine levels in the striatum.

With the impulse flow non-existent in these preparations the effects of neuronal feedback loop are eliminated and any drug induced alterations must be mediated by another mechanism.

It has been shown that apomorphine prevents the increase in dopamine levels, (164, 296) induced by cessation of impulse flow and that the increase is restored by a combined treatment with haloperidol and apomorphine. Therefore it was proposed that the effect is mediated by a presynaptic dopamine receptor, (11).

B2.1 TYROSINE HYDROXYLASE ACTIVITY.

The accumulation of Dopa after the inhibition of dopa-decarboxylase is considered as an index of the activity of (TOH) tyrosine hydroxylase, (11).

Since after the axotomy of the nigrostriatal fibres, the accumulation of dopa is increased, it was concluded that the activity of TOH is enhanced.

The control of TOH cannot be exerted in this case by end product inhibition since according to this idea, when there is a decreased impulse flow and less transmitter is used, the synthesis should be slowed.

Thus the dopamine system appears to respond differently when compared to other monoamine systems, (11). In particular, an inhibition of impulse flow causes a rapid increase in the transmitter contents of its terminals. In similar conditions, the synthesis of norepinephrine for instance does not increase, (11, 49, 177).

When the nigro-striatal fibres are stimulated there is a stimulus dependent increase in the accumulation of Dopa, (255). And this increase in TOH activity persists for about 15 minutes after cessation of stimulus.

The kinetics of the enzyme are also affected; after stimulation, there is an increase in the affinity of TOH for the pterin cofactor (BH_4, an electron donor), and an decrease in the affinity for the end product inhibitor, dopamine, (256).

Therefore, in these conditions the enzyme is more active to produce more Dopa and at the same time less liable to be inhibited by dopamine/

dopamine if it accumulates.

After impulse flow inhibition, there is also an increase in TOH activity, but the kinetics of the enzyme are different from those after stimulation. TOH has a marked increase in its affinity for tyrosine and pterin cofactor and a marked decrease in its affinity for dopamine. Therefore, in this case, although the enzyme is more active and produces more Dopa, it is not inhibited by its end product causing an increase in dopamine.

Thus it appears that a blockade of impulse flow leads to a change in the physical properties of the enzyme molecules; the problem is to know the mechanism responsible for this change.

A possible mechanism has been proposed based on the idea that adenyl cyclase could be the dopamine receptor.

B2.2 ADENYL CYCLASE AS THE DOPAMINE RECEPTOR.

This enzyme forms cyclic adenosine monophosphate (cAMP) from adenosine triphosphate, (ATP).

It was first observed that dopamine, which was known to hyperpolarize the post ganglionic neurons of the superior cervical ganglion also caused an increase in ganglionic cAMP (198). Moreover it was found that cAMP is able to mimic the hyperpolarizing action of dopamine, (199).

Using homogenates of the ganglion, it was shown that dopamine stimulated the adenyl cyclase, (162). This occurred at very low concentrations of dopamine, suggesting that the effect was specific, (162, 126). Homogenates of rat striatum also contain dopamine sensitive adenyl cyclase which has several of the properties of the striatal dopamine receptor, (126, 12, 161). For example, the stimulatory/

stimulatory effect was mimicked by very low concentrations of apomorphine and prevented by antipsychotic drugs (149, 127, 47).

A number of neurotransmitters besides dopamine, like serotonin and norepinephrine, (125) increase the cAMP content of brain slice. Such increase apparently results from stimulation of separate and distinct receptors for dopaminergic, serotoninergic and noradrenergic compounds.

Through the use of specific blocking agents it appears that these compounds have independent sites of action, (125).

It has been observed that an enzyme, a protein kinase, that catalyses the phosphorylation, by ATP, of phosphorylase kinase, is dependent on cAMP; therefore the level of phosphorylation of substrate proteins, is mediated indirectly by cAMP. This has led to the idea that cAMP-mediated phosphorylation of proteins may be involved in neurotransmission. It has been proposed that this protein phosphorylation may be involved in changes in synaptic permeability, transmitter synthesis and phosphorylation of myelin basic protein, (211).

Adenyl cyclase can be stimulated by low concentrations of calcium, suggesting that it may have a role in its regulation. It could be possible that depolarization resulting in an influx of calcium could affect cAMP mediated processes such as protein phosphorylation.

B2.3 A MODEL TO EXPLAIN TYROSINE HYDROXYLASE REGULATION. AN ALTERNATIVE EXPLANATION, TO THE FEEDBACK HYPOTHESIS (1).

Roth, in 1975, (256) proposed a model for tyrosine hydroxylase activation. An increase in impulse flow leads to an increase in calcium influx. By "some" calcium dependent

dependent reaction, the adenyl cyclase in the presynaptic membrane is activated increasing the levels of cAMP, which in turn activates a protein kinase which could phosphorylate TOH converting it into a kinetically activated form, or phosphorylate a protein which then serves as an activator of TOH.

It has been postulated that when the impulse flow is decreased, calcium influx is diminished triggering a reversable change in the conformation of TOH causing the enzyme to have an increased affinity for tyrosine and pterin cofactor and a decreased affinity for end product inhibition.

To support this model, in soluble preparations of TOH obtained from rat striatum, it was found that a decrease in the concentration of calcium resulted in an enzyme which has similar properties to the enzyme after inhibition of impulse flow (256). This is in agreement with the increase in dopamine synthesis observed in the striatal slices when calcium was removed from the medium, (131).

An indirect way of testing this model was based on the idea that a receptor blocker will remove any modulatory influence on TOH by the receptor, leading to an enhanced activation of TOH. The hypothesis was tested stimulating the nigro-striatal pathway in intact brains at a frequency, and for a period of time, such that no further activation of TOH could be obtained by a possible feedback loop. The stimulation alone produced a TOH whose kinetics showed an increased affinity for end product inhibition. The stimulation plus haloperidol causes an increase in the stimulation induced activation of TOH; that is, it generates a form of a TOH with a decreased affinity for end product inhibition, (131). The opposite effects were obtained after

apomorphine/

apomorphine administration (133).

In this model, the more elaborated part is the effect proposed after an increase in impulse flow leading to an increase in the influx of calcium which by an unknown process increase the kinetics of TOH. Not explained in such detail are first, the effect of a decrease in impulse flow which by the lack of calcium influx also alters the kinetics of TOH but with an affinity for end product. And second, in an imprecise way, dopamine agonists and endogenous synaptic dopamine are believed to exert a braking effect in the synthesis of dopamine acting on presynaptic receptors. Receptor blockers, in contrast are assumed to remove that braking effect increasing dopamine synthesis.

This model is supported by the experiments in striatal slices (section B1). However the direct and convincing demonstration of this model has not been done, and until then it can only be considered as a model. Especially since Lovenberg et al (192) have been unable to find the decrease in the affinity of TOH for dopamine, after a reduction in the concentration of calcium.

B3 PRE- AND/OR POST-SYNAPTIC RECEPTORS.

Antipsychotic drugs concentrate in cell membranes since they are highly surface active and fat soluble, (150). This made it difficult to estimate specific binding sites, until an antipsychotic drug, butaclamol, which has an active and an inactive enantiomer, was used. In this way the binding is calculated as the difference between the amount of radioactive haloperidol bound in the presence of the inactive and the active butaclamol, (187).

With/

With these studies, surprising results have been obtained. The simplistic idea of a single receptor onto which dopamine (agonists and antagonists) bind exclusively has been questioned. It seems that two antipsychotics, haloperidol and spiperone bind to different sites (187), perhaps involved not only with dopamine but with serotonin. Spiperone binding sites seem to be relatively more influenced by catecholamines, (187, 181). In the frontal cortex, specific binding of labelled spiperone or labelled LSD (lysergic acid diethylamide - a possible serotonin receptor blocker) was inhibited more by serotonin like compounds than by dopaminergic agonists, (187). However serotonin is less potent than dopamine in inhibiting labelled dopamine binding in striatum, (45, 187).

If the concentration necessary to compete for 50% (IC-50) of the labelled dopamine binding sites is compared with the concentration necessary to produce IC-50 of the adenyl cyclase activity, it is observed that the affinities of catecholamines for labelled dopamine binding sites in the rat are greater than their potencies in enhancing dopamine sensitive adenylate cyclase, and by contrast, antipsychotic drugs, are more potent as inhibitors of adenylate cyclase than of dopamine binding sites in the rat, (45, 92). The IC-50 for the antipsychotic drugs to compete for labelled haliperidol binding sites is different from the one for labelled dopamine, (265).

These discrepancies point out that perhaps agonists and antagonists are not binding to the same number of receptors, or that not only adenylate cyclase is the dopamine receptor.

Lesions of dopamine cells with 6-hydroxy-dopamine, (6-OHDA, which produces degeneration of dopamine and noradrenaline containing neurons, 284), are supposed to cause degeneration of the nigro-striatal pathway

and its terminals. This produced no difference in the labelled dopamine binding sites in the striatum compared with the unlesioned side (45). and the adenyl cyclase activity was still observed on both sides (206). An increase in the receptor affinity in the lesioned side could explain the lack of difference between the control and lesioned sides. But also this can be taken against adenyl cyclase as the dopamine receptor.

A compound with similar excitatory properties to the amino acid sodium glutamate, called kainic acid, (229), when injected into a brain nucleus, produces degeneration of the cells whereas axons passing through or terminating in the region appear to be unaffected, (262). Its neurotoxic effects seem to be similar to the action of sodium glutamate, which may be due to its depolarizing action, (229).

Injections of kainic acid into the striatum, produced a significant decrease in adenylate cyclase after 48 hours, whereas the binding of labelled haloperidol was only reduced by 20%. If a lesion of the cerebral cortex followed, the binding of labelled haloperidol decreased another 50% indicating that most of the striatal haloperidol receptor sites are probably localized to axons of cerebral cortical afferents, whereas dopamine sensitive adenylate cyclase is confined to neurons intrinsic to the striatum, (263).

All this evidence points out that the problem of the dopamine receptor is not as simple as it was thought and that it still remains to be established if the adenyl cyclase is part of the dopamine receptor and if it is the only one. It also suggests that there are presynaptic receptors in the terminals of cortical neurons as well as the dopamine terminals in the striatum.

Carlsson/

Carlsson (52, 51), has proposed that pre- and postsynaptic receptors may be distinguished by the dose relationships for different actions of apomorphine. Low doses of apomorphine, produce an inhibition of Dopa synthesis which will not have any observable effect on a behaving animal, while a dose ten times higher will produce several alterations in its motor behaviour. Therefore he proposes that there is a preferential activation of presynaptic receptors which are the ones that respond to low doses of the drug, and only when it is increased, postsynaptic receptors are activated with their consequences on motor behaviour.

2C. DOPAMINE-ACETYCHOLINE-GABA INTERACTIONS IN A FEEDBACK LOOP.

Using immunohistochemical techniques with fluorescin labelled antibodies against choline acetyltransferase, (ChAT) it has been possible the localise some ChAT containing neurons, (141, 201). Labelled cells and dendrites were observed in the striatum, (141) and after 6-OHDA lesions, in substantia nigra, degenerating dopamine boutons were observed on dendrites or spines of labelled cells for ChAT, (141) indicating that dopamine cells synapse on ChAT containing cells. Moreover a 'pile-up' of the enzyme is not observed in the striatum after lesions of the fibres leaving the nucleus, an effect found in ChAT containing cells when their axons are cut, (141). This indicates that these cells are contained within the nucleus as interneurons.

It has been shown that Acetylcholine release and turnover is increased in the striatum by antipsychotic drugs, and decreased by apomorphine or L-Dopa, (137, 194, 18). In other brain areas it does not change, (18, 60, 137), indicating that probably it is in the striatum/

striatum where dopamine terminals inhervate a relevant number of cholinergic neurons.

After a blockade of Ach synthesis with hemicholinium-3, the rate of disappearance of Ach is increased by antipsychotic drugs, (137). It appears then, that the decrease in Ach levels after antipsychotic drugs could be due to an increased rate of utilization of striatal Ach. It thus seems that antipsychotic drugs enhance and dopamine agonists decrease the activity of striatal cholinergic neurons.

Following the administration of neuroleptic agents, the enhanced Ach liberation could reflect disinhibition of cholinergic neurons due to removal of an inhibitory dopaminergic input.

Electrical stimulation in substantia nigra, reduced the liberation of Ach in striatum, (18), supporting the idea of dopamine as an inhibitory input into Ach cells there. This input seems to be tonic, since transection of the dopamine pathway results in increased spontaneous Ach release in the striatum, (137).

2C.1. ALTERNATIVE EXPLANATION TO THE FEEDBACK HYPOTHESIS, (11).

At this stage the results could be summarized by saying that there appears to be an inhibitory synapse of dopamine containing terminals onto Ach containing cells in the striatum, however, although this could be the case, other results complicate this oversimplification.

Cholinomimetic agents (like, Oxotremorine, physostigmine or carbachol) increase dopamine release, measured by means of a push-pull cannula, (19) or as an increase in labelled dopamine formed from labelled tyrosine, (120). In the same way, cholinergic blockers (muscarinic and nicotinic) decrease the release of dopamine, (19, 120). Based on this/

this evidence it has been proposed that the Ach containing neurons on which dopamine terminals synapse, send in turn a collateral which synapses into the dopamine terminal, facilitating dopamine release, (19, 120, 20). In accordance with this it was found that atropine blocks the haloperidol induced increase in dopamine (19). (Fig.1).

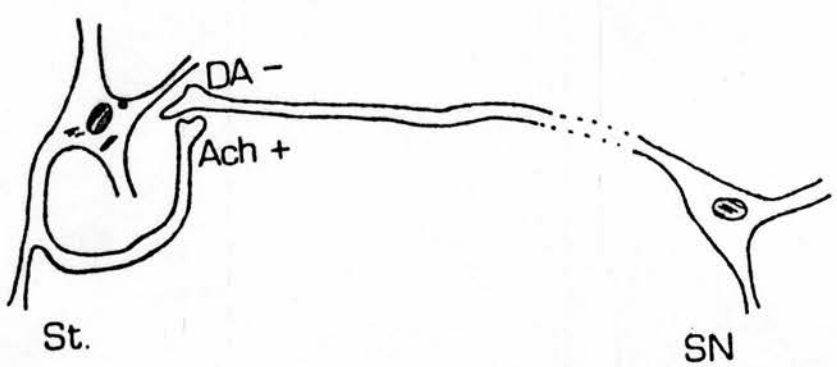

Fig. 1 HYPOTHETICAL REPRESENTATION OF A DA-Ach INTERACTION IN THE STRIATUM.

(-) inhibitory, (+) facilitatory influence, ST. = Striatum, SN = Substantia Nigra.

The effects of manipulating GABA on dopamine release are contradictory. While it has been shown that intraperitoneal injection of picrotoxin (GABA, receptor blocker) increases dopamine release, (282), it has also been found that it decreases the release of newly synthesized labelled dopamine from labelled tyrosine (121). GABA and Muscimol (a GABA agonist) enhanced the spontaneous release of dopamine. It has been proposed that Ach collaterals synapsing on dopaminergic terminals have GABA receptors that when activated in the presence of GABA increase the release of Ach, (121), (Fig.2).

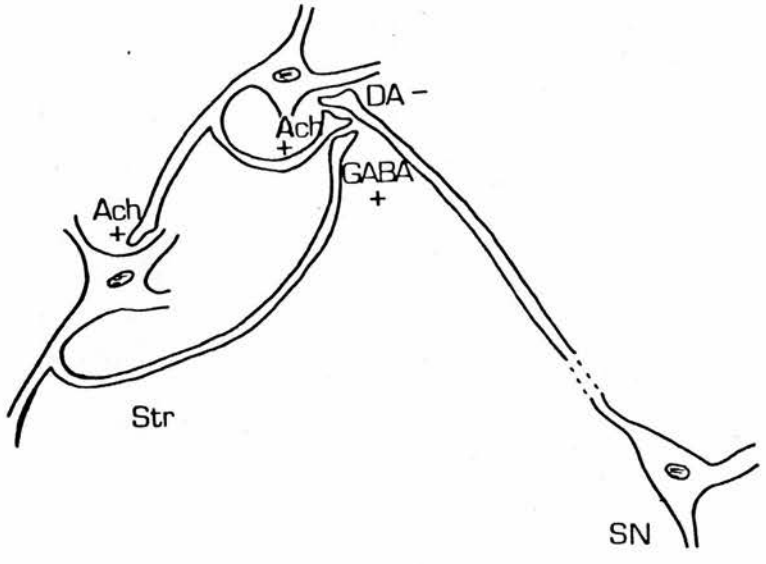

Fig.2 HYPOTHETICAL REPRESENTATION OF DA-Ach- GABA INTERACTION IN THE STRIATUM.

Symbols as in Fig. 1.

The models of presynaptic control of dopamine release are not explained in such detail as to include the mechanisms involved for such control, however there is the implicit idea of a depolarization or a hyperpdarization effect.

If acetylcholine is considered to facilitate the dopamine containing terminals with influx of calcium, the TOH kinetics will be altered in such a way that the increase in dopamine release after haloperidol will not be observed, (Section, 2.B, 2, 3). Of course, since both are models they need not necessarily both agree.

If depolarization is still considered as a possible mechanism, the first thing that can be concluded is that Ach is producing presynaptic inhibition, and therefore decreasing the amounts of transmitter available. Which does not fit with the idea of Ach producing an increase in dopamine release after haloperidol.

Hyperpolarization of the presynaptic terminal perhaps could explain the results, since it has been shown that even though it is more difficult to reverse the membrane potential, when it is reversed during an action potential, it releases more transmitter, (91).

Examined carefully, the models of dopamine control by Ach and GABA interactions somehow do not seem to be very clear. Evidence for Ach-GABA interactions is also needed.

2.D DENDRITIC CONTROL OF DOPAMINE RELEASE.

By the evidence presented so far, while it cannot be excluded completely/

completely that the receptors involved in the regulation of dopamine release are located at the postsynaptic cell bodies, there are also observations indicating that there is a local receptor-mediated feedback mechanism, confined to the dopaminergic nerve terminal area.

It has been found (Section 3, E.3) that nigral dopaminergic cell bodies respond to locally applied dopamine and apomorphine, with a decreased firing and that this effect can be blocked by haloperidol, (39, 41). Since nigral dopaminergic cells do not seem to receive any input of dopaminergic nerve terminals, it was suggested that the dopaminergic receptors possibly located at the cell bodies and at nerve terminals "serve as sensors for the neuron's own transmitter in the medium surrounding it", (53). The term autoreceptor has been proposed (52), which includes the so called presynaptic receptor at terminals in the striatum, as well as the receptors located in the cells bodies and dendrites in substantia nigra.

On the other hand, varicose dendrites extending to the substantia nigra reticulata were observed by fluorescence microscopy, (31). Furthermore, the fluorescence disappeared after injecting reserpine 12-33 hours before killing the animal and was increased after incubation of the slides with dopamine, (31). This indicated that probably the dendrites and cell bodies take up, store and release dopamine, as do the axon terminals. To strengthen this point, it was found that in slices from rats' substantia nigra, labelled dopamine was taken up probably by dopamine dendrites and cell bodies, (119), and possibly by axon collaterals too. To minimize the possible contribution by cell bodies, the substantia nigra compacta was separated from the substantia nigra reticulata. After incubation in dopamine, both areas showed an increase in/

in their dopamine content, (73). Suggesting therefore a dopamine take up by dendrites as well as cell bodies.

Taking advantage of the ability of nigral tissue to incorporate labelled dopamine, it was shown that once it is taken up, it is also spontaneously released, (73). After exposure to potassium, (which stimulates calcium dependent transmitter release (32), an efflux of labelled dopamine was observed, providing the superfusion medium contained calcium (73,119). This shows that dendrites in the substantia nigra reticulata as well as dopamine cell bodies in substantia nigra compacta, incorporate and release dopamine.

With this evidence it was thought that if dendrites are liberating their transmitter, and if the concept of autoreceptors is true, an obvious place for those dendrites to act is on the cell bodies or dendrites of neighbour dopamine cells (lateral inhibition) or on the same cell (auto-inhibition).

The idea of lateral inhibition was strengthened when evidence for dendro-dendritic associations containing vesicles (139), was taken into account. However, in another similar study, (73) no morphological signs of synaptic conctacts, (such dense projections, membrane attachments, vesicles or membrane thickenings) were found between dendrites. On the other hand even though dendro-dendritic appositions were frequently observed, dendrites were also found to associate with blood vessels, (31, 73). But again no evidence was found of synaptic contacts, (73). Dopamine released from dendrites could act on blood vessels and influence microcirculation in the substantia nigra, but so far there is no evidence to support such hypothesis.

The lack of consistent evidence about dendro-dendritic synapses, weakens/

weakens the point that dendrites may act on neighbouring dendrites. And this is emphasized when studies on adenyl cyclase are reviewed.

If the adenyl cyclase is considered to be associated with the dopamine receptor (Section 2, B.3) and if the concept of autoreceptor is true, there must be a dopamine-sensitive adenyl cyclase in substantia nigra. This appeared to be the case when in homogenates of the substantia nigra reticulata, a dopamine-sensitive adenyl cyclase was found, (163). However, after 6-OHDA injections in substantia nigra, (which produces degeneration of dopamine and noradrenaline containing neurons, 284), no difference was found in the dopamine stimulated adenyl cyclase from intact and injected sides, (163, 236, 117). Therefore the dopamine receptor did not appear to be in the cell bodies or dendrites of dopamine neurons.

In order to find out where the adenyl cyclase was located, different experiments were done. Hemisections between the substantia nigra and striatum, (267, 117), lesions in the globus pallidum, (236) or in the striato-nigral pathway, (267), indicated that the adenyl cyclase was present in the afferent fibres interrupted by the lesion, since the dopamine-sensitive adenyl cyclase was markedly decreased after the lesions.

Only the afferents coming from the striatum and globus pallidum seemed to be involved, since a hemisection posterior to substantia nigra, interrupting the afferent serotoninergic fibres from the raphe nuclei, did not alter the adenyl cyclase in substantia nigra, (117).

With this evidence, it has been suggested that dopamine released from dendrites may act on the afferent fibres innervating substantia nigra and controlling the release of their transmitter among nigral cell/

cell bodies or dendrites, (73, 236, 117, 267).

D.1 AN ALTERNATIVE EXPLANATION TO THE FEEDBACK HYPOTHESIS, (III)

D.1.1 GABA CONTAINING AFFERENTS.

Since GABA afferents from the striatum have been described (Section A.2) an alternative model for the mechanism of action of the antipsychotic drugs is proposed, by saying that dopamine interacts with the adenyl cyclase receptor in GABA afferents, in such a way that when dopamine is present, GABA is released inhibiting the neurons, and when a dopamine blocker is given, then GABA is not released and the dopamine neurons are disinhibited, (117, 267), (Fig.3A).

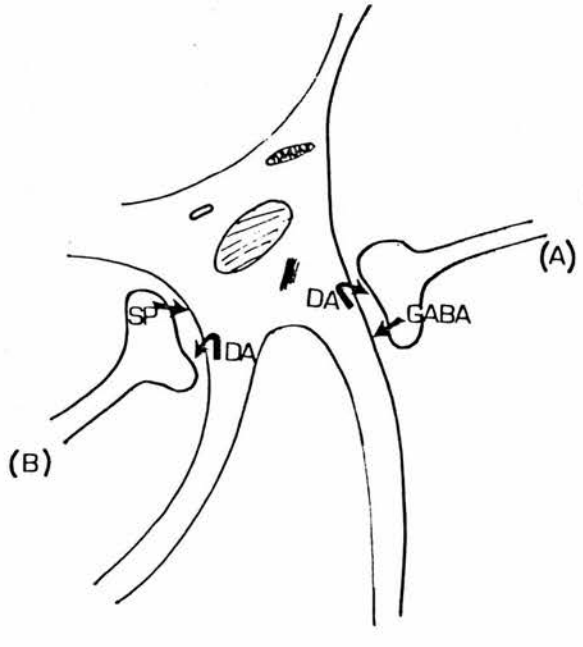

Fig.3. HYPOTHETICAL REPRESENTATION OF GABA-DOPAMINE (A) AND (B) SUBSTANCE P-DOPAMINE INTERACTIONS IN SUBSTANTIA NIGRA.

D.1.2. SUBSTANCE P CONTAINING AFFERENTS.

Substance P is an undecapeptide, highly concentrated in the dorsal horn of the spinal cord of cats and rats, and an excitant of motoneurons, (94). It has been found in different parts of the brain, (159) and a stimulus evoked release of Substance P has been demonstrated from rat hypothalamic slices, (152) making it a candidate for a neurotransmitter in the central nervous system, (74).

The substantia nigra has one of the highest concentrations of substanceP, and hemisections or lesions interrupting the pathway from striatum and globus pallidus to substantia nigra produce a decrease in the amount of substance P in substantia nigra which is correlated with the decrease in GAD activity that these lesions also produce, (117, 236 and Chapter II, Sections D.1 and E.4). Electrothermic lesions in the striatum and globus pallidum, also produce a significant decrease of both GAD and substance P, (160). Immunohistochemical techniques showed that some cells in the globus pallidus and striatum were substance P positive, (160). Therefore, since pathways containing substance P are also interrupted in the studies showing adenyl cyclase in the afferents to substantia nigra, a modulatory action of dopamine on these fibres is also mentioned, (236, 117), (Fig. 3B).

This model is not very specific, and can be subjected to similar criticisms to the model of Ach, GABA and dopamine interactions, (Section 2.C); it leaves the modulatory action of dopamine, in this case, to an unknown mechanism which facilitates or inhibits GABA or substance P.

It/

It is based on the activity of adenyl cyclase which may not be the only dopamine receptor if it is in fact the receptor, (Section B.3).

Even though this evidence contributes to the idea that there is also a release and turnover of dopamine in substantia nigra, the model could not be used alone to explain the changes in dopamine turnover in the striatum, since it could not explain the effects of nerve impulse blockade (Section B.2) or in vitro studies of striatal slices, (Section B.1).

2.E PREVAILING INTERPRETATIONS.

It is not possible to exclude completely with the evidence presented so far, that fibres to substantia nigra do not exert any effect on dopamine cells, but it is also clear, that the striatum and the substantia nigra, could regulate their synthesis of dopamine if left without any communication from each other.

Several hypothesis have been tested in order to find how is it that these structures regulate the synthesis of dopamine, and so far none of them seem to be completely satisfactory.

A carefully elaborated model is the one presented by Roth, (Section 2.B.2), however its main criticism could be that other researchers have not been able to corroborate his results. Furthermore it is based on the activity of an adenyl cyclase as the presynaptic dopamine receptor whose existence is not at all clear, (Section 2.B.2).

A much more imprecise model is the one involving interneurons, dendrites and axon collaterals of Ach, and GABA containing cells, (Section 2.2.C). No doubt that these interrelations may exist but in this case more information is needed about these interconnections and their/

their effects, before any conclusions can be drawn. It is however a good approach to the problem, since it conceives the system in a more realistic way.

The evidence showing dopamine release in the substantia nigra, and its possible control over the afferents in the substantia nigra, (Section 2D) again shows how complicated this interaction can be. It also points out that there might be, at least, two mechanisms for the control of dopamine turnover, one located in the substantia nigra, and the other in the striatum, so that if the system is disconnected it still can work independently.

3. ELECTROPHYSIOLOGICAL EVIDENCE: RECIPROCAL RELATIONS BETWEEN THE STRIATUM AND THE SUBSTANTIA NIGRA.

According to the feedback hypothesis, dopamine exerts an inhibitory action on striatal cells, and these in turn, decrease dopamine neurons activity in the substantia nigra; process which is reversed after antipsychotic drugs, (Section 2.A). This hypothesis inspired many electrophysiological experiments, some of which, together with others not directly related to this hypothesis, will be examined in this section.

3.A METHODOLOGICAL CONSIDERATIONS.

Before studying in detail the results of these electrophysiological interelations, some problems which make the interpretation of the results difficult, must be mentioned.

1) Those experiments using electrical stimulation of the striatum or substantia nigra, cannot completely guarantee that the stimulation is localized/

localized, not only by the kind of electrode or amount of current used, (242), but also by the spread of current to other areas. Attempts to rule out stimulus spread, have included lesions of areas around the stimulating site (113, 148, 113), arrays of stimulating electrodes, so that the effects of activating adjacent tissues could be compared with those elicited by nigral stimulation, (148, 65); and manipulating electrode depth, (148). In spite of all these techniques, one should bear in mind that the stimulation may excite other neurons of which only a portion actually projects to the recorded site, and that polysynaptic circuits may be involved for long latency responses; or antidromic activation of other fibre systems may be confused with the effects of orthodromic stimulation, (249, 306).

2) A problem frequently found in electrophysiological studies, of the striatum, is the paucity of spontaneously discharging cells; in some cases, L-glutamic acid or dl-homoeysteic acid (amino acids which excite cells in the nervous system, 270) is iontophoresed in order to increase the firing rate of these cells, (145, 64); in those cases the actions of other experimental manipulations must be interpreted against that artificial background of the response.

3) Some of those "silent" neurons are also excited by stimulation of the thalamus, cortex, (113, 145), or from within the caudate, near the recording electrode, (195); such procedures, are useful for studying a selected group of neurons, however, these cells may not be representative of all the neurons innervated by substantia nigra afferents, in this particular case.

4)/

4) Intracellular recordings have the advantage of recording membrane potential changes, even when action potentials are absent, although they introduce a probable sampling bias in favour of larger cells.

5) Studies from the substantia nigra often fail to show a detailed anatomy of the recording or stimulating sites, therefore the electrode could be in the pars compacta or pars reticulata, which could make the interpretation of the results different if the anatomical connections are taken into account, (Section 1C.)

6) In some animals the cortex overlying the caudate nucleus is removed, (171, 113, 170). In these cases although the placement of the recording electrode may be facilitated, and the micropipetes block less, the activity of the cells may not be the same in view that cortical projections to this area are destroyed, and spreading depression is also produced, (110, 166).

7) In general it is important to point out that it is necessary to: A) make a systematic identification of cells in terms of the input they receive from different structures, as well as its nature, (mono or polysynaptic) and B) do combined pharmacological and electrophysiological experiments.

3B. NEURONAL ACTIVITY IN THE STRIATUM AFTER STIMULATION OF THE SUBSTANTIA NIGRA.

Even before the pathway from the substantia nigra to the striatum was anatomically established, electrophysiologists were trying to determine whether or not they could find responses in the striatum after/

after substantia nigra stimulation, (113, 145).

Most of these experiments, were done in cats, some immobilized with gallamine triethiodide, (148, 101), or with a section through the midbrain (cerveaux isole preparation), (203, 304, 113, 64, 302), after which only local anaesthesia is required.

When the activity of cells in the caudate nucleus or putamen was increased by iontophoresis of amino acids (Section 3A), the stimulation of the substantia nigra, produced an inhibition of the firing rate which lasted approximately 40-60 msec, (304, 203, 64). A similar experiment done in immobilized rabbits reported a longer inhibition, 80-350 msec, (145). An increased discharge has also been observed after the inhibition, (145, 64, 113).

A focal negative potential concomitant to the stimulation is sometimes described; this potential has a peak latency of 2.5-6 msec, and its amplitude is variable and dependent on stimulus parameters, (101, 113, 302, 304). It probably indicates a current flow from a population of cells near the recording electrode, possibly innervated by the stimulated fibres.

Sometimes, an action potential is associated with this focal potential, (302, 101, 304, 113) its latency varies from one experimental report to the other ranging from about 5 msec, (302, 304) to 16-20 msec, (101, 113). This spike fails to follow high frequencies (50-100 p/sec) suggesting it is orthodromic, confirmed by the fact that it does not collide with spontaneous spikes, (105); spikes of latencies less than 4 msec, have been considered as antidromic potentials evoked in striato-nigral fibres excited by nigral stimulation, (113). The conduction velocity calculated for this action potential, accounting

for/

for one synapse, falls between the range of conduction of non-mylinated fibres, (0.5-2.0 m/sec), (105, 113, 170) which agrees with the anatomy of this pathway, (Section 1). The orthodromic spike produced by nigral stimulation, contrasts with the inhibition observed after an induced excitation by iontophoresis of amino acids, and even in these conditions, in some cases, a further excitation has been observed after the stimulation, (64). It has been reported that this spike is blocked by intravenous injection of haloperidol in anaesthetized rats, (240) or by superfusion of haloperidol through a cup placed on the surface of the caudate nucleus of cats, (104), indicating that this potential is mediated by dopaminergic receptors.

Some spontaneously active cells have been excited, inhibited, or unaffected by nigral stimulation, (101), suggesting that not all striatal cells are innervated by dopamine containing nigral afferents and that even those which seem to receive an input from substantia nigra, show different responses. In order to explain this discrepancy, two dopamine receptors have been proposed, one mediating inhibition and the other excitation, (302, 203); it has also been proposed that there are two kind of cells, some inhibited by nigral fibres, and sensitive to iontophorized amino acids, and some excited by nigral stimulation which are not spontaneously active and have a high safety factor following high frequencies of stimulation, (up to 300 p/sec, 105).

Intracellular records of caudate cells also show different excitatory or inhibitory post-synaptic potentials (EPSP's and IPSP's) to nigral stimulation. These span the whole range from IPSP's alone, through EPSP-IPSP combinations, to EPSP's alone (148, 113, 171, 38). The EPSP-IPSP arrangement seems to predominate, (113, 38, 171); the IPSP's/

IPSP's recorded show a longer hyperpolarization period when compared to similar responses produced by cortical or thalamic stimulation, (148) and frequencies of stimulation higher than 10 p/sec led to hyperpolarizations even when responses to single stimuli produced EPSP's, (148). This agrees with the observation that a longer lasting inhibition of induced discharge is observed after repetitive stimulation, (64).

The EPSP-IPSP sequences have been explained as an initial excitation produced by the stimulated input, followed by inhibition generated from within nucleus, mediated by the interneuron collaterals, (148, 38).

The relationship between the stimulated fibres and the recorded cells has been considered as monosynaptic, because the EPSP's keep a constant rising point regardless of the stimulus intensity or frequency, (171, 113). This monosynaptic input appears to be on interneurons since antidromic stimulation of cells whose axons leave the striatum (striato-nigral pathway) shows that these do not have monosynaptic potentials, (170). Apparently these interneurons do not only receive inputs from the substantia nigra but from the motor cortex and the centromedian and parafascicular nucleus of the thalamus, (171, 38). Inputs which seem to converge onto a single neuron as there was a summation of their corresponding EPSP's after combined stimulation of these areas, (171).

Without taking into account the possibility that these results are due to antidromic stimulation of striato-nigral fibres, (249, 306), the most consistent results could perhaps be summarized as follows: it appears that the substantia nigra stimulation most frequently induces an excitation followed by an inhibition probably on caudate interneurons,/

interneurons, represented by EPSP's-IPSP's sequences. Although in spontaneously or induced active cells, it produces an inhibition, suggesting at least two kinds of neuronal populations from electrophysiological criteria.

3C. ELECTROPHYSIOLOGICAL RESPONSES OF CAUDATE NEURONS TO DRUGS.

C1. DIRECT APPLICATION OF DOPAMINE.

Dopamine ionotophoretically ejected in the caudate nucleus of anaesthetized or decerebrated cats and monkeys produces a similar mixture of responses. It decreases the firing rate of spontaneously or induced active cells, (102, 65, 261, 268, 33, 203, 303). A small but consistent group of cells is facilitated, (203, 303) many neurons do not respond, and a few (14-18%) show a combined effect, with an initial inhibition followed by an excitation, (303, 302).

The effects of iontophoretic dopamine on caudate neurons responding to nigral stimulation have been reported only in a few instances (303, 102, 65, 261), in these cases both dopamine and nigral stimulation reduced the firing rate of cells (102, 65). Also iontophoretically applied dopamine potentiated the inhibition produced by electrical stimulation (261). Units which responded to stimulation by evoked spikes or increased firing rates, have been reported to be depressed, (203), unresponsive, (102) or show mixed responses (65) to dopamine. Some of the confusion in this point, may be due to different experimental designs (e.g. anaesthesia, vrs decerebration, etc) and different recording sites.

If the depressant effect of nigral stimulation and iontophorized dopamine,/

dopamine, share a common mode of action, it should be possible to block them both, by a blocking agent. However, adrenergic alpha-receptor blockers, (phenoxybenzamine and phentolamine) antagonized the action of dopamine, but not the effects of nigral stimulation (203, 304, 65). Chlor promazine, iontophorized to the caudate nucleus of anaesthetized monkeys (303) abolishes the effects (facilitation and inhibition) of iontophorized dopamine. The same has been observed in recordings from the putamen of anaethetized or decerebrated cats, (302, 304), but no stimulation of the substantia nigra was done in these cases. Therefore it remains to be established if both, nigral stimulation and iontophorized dopamine have a common mode of action.

C2. EFFECTS OF ACETYLCHOLINE (Ach) AND GAMMA-AMINO-BUTYRIC-ACID (GABA).

As has already been mentioned, (Section 2.A.2; 2.C), there are GABA and Ach containing neurons in the striatum, possibly involved in a feedback control of dopamine synthesis. Electrophysiological studies have tried to clarify the effect of these transmitters on cell activity in the caudate nucleus, however the results do not seem to contribute to this aim.

Iontophorized Ach, mainly increases the firing rate of spontaneously active cells in the caudate nucleus, (29, 28, 268, 33), although less than 10% of the cells showed an inhibition or did not respond, (268, 33). Anaesthetics (chloralose, or pentobarbital) decreased the facilitatory effect of Ach, (33). A lesion of the nigro-striatal pathway enhanced the effect of Ach, (268) suggesting that after dopamine denervation, this population of recorded cells became more sensitive to Ach.

Intracellular recordings of striatal cells in immobilized rats, (24), show/

show that iontophorized Ach produces EPSP's with a subsequent increase in the firing rate. GABA iontophorized into the striatum of rats, abolished spontaneous activity recorded intracellularly and induced IPSP's (23, 25); picrotoxin reversed its effect, (23).

These results give some evidence about the intrinsic role for Ach and GABA in the striatum proposed with the biochemical results, (Section 2.C) but much more experiments are needed to confirm the relations proposed by the biochemical models.

C3. PREVAILING INTERPRETATIONS.

To make a summary of these electrophysiological results without eliminating potential relevant information is almost impossible, therefore, this is only to point out the most consistent results from different experimental reports.

It appears that in agreement to what is proposed in the feedback hypothesis stimulation of the substantia nigra leads to inhibition of spontaneous or induced activity of caudate cells, (304, 203) and that this effect is reproduced by the direct application of dopamine, (102, 261, 203).

In some cases stimulation of the substantia nigra leads to facilitatory effects on caudate cells, producing spikes or increasing their firing rate, (302, 105, 113). Confusing results are obtained when dopamine is iontophorized, sometimes these cells are inhibited, facilitated or do not respond, (203, 102, 65). In order to explain the contradictory facilitation which does not agree with the supposed effect of dopamine, two different kinds of neuronal populations have been proposed, possibly having two different receptors, (203, 302). The

spike/

spike produced after nigral stimulation seems to be mediated by dopaminergic receptors, since it is abolished by haloperidol, (104, 249). This action potential has allowed the calculation of the conduction velocity, suggesting that the excitatory nigro-striatal pathway is slowly conducting, (0.5-2.0 m/sec).

In view of the possible presynaptic regulation of dopaminergic terminals, (Section 2.C), as well as the apparent complicated connections inside the nucleus, it seems naive to look at the effect of drugs on several cells as if they belong to the same population. Probably most of the conflicting results are due to this approach.

3D. NEURONAL ACTIVITY IN THE SUBSTANTIA NIGRA AFTER STIMULATION OF THE CAUDATE NUCLEUS.

Extracellular recordings of substantia nigra cells after caudate stimulation with a single pulse, produces in most cases, (60-90%) an inhibition of the firing rate of spontaneously or induced active cells, (305, 88, 204, 103, 107, 134, 108). These results are common to all the experimental animals, (e.g. rat, cat, monkey) or their experimental condition, (e.g. anaesthetized or cerveaux isole). In a smaller but consistent number of cases, (always less than 50%), an increase in the firimg rate preceded or followed the inhibition, (88, 103, 107, 108, 113).

A positive field potential is usually recorded in cats and rats, which correlates with the onset and duration of the inhibition of the firing rate, (88, 305, 204, 241), probably related with focal current movements.

There is a disagreement between the experimental reports about the duration of the inhibition, but this would be expected as inherent changes/

changes in the experimental conditions; for instance, it has been demonstrated that in rats, the latency of the inhibition shifts from 5.4 to 8.9 msec from urethane to pentobarbitone anaesthesia, (88).

Intracellular recordings in cats, agree with the results obtained extracellularly; in most cells (more than 50%), IPSP's are observed following stimulation of the caudate nucleus, (305, 114). In a few cases, (less than 15%) EPSP's are recorded alone, (114) or preceding the IPSP's (305). It has also been reported that sequences of EPSP-IPSP's as a result of repetitive stimulation, (8 p/sec) in the caudate nucleus of cats, are obtained in the substantia nigra as the rule and not the exception, accompanied by action potentials, (113). Due to the constant shape and latency of the PSP's and the lack of temporal facilitation, these are considered as monosynaptic potentials, (305, 114, 113).

Some of these PSP's are reported to have latencies between 3-5 msec and some between 10-15 msec, (305, 113, 114). It has been proposed that fibres of different diameter and conduction velocities convey the impulses from the caudate to the substantia nigra; conduction velocities of about 5 m/sec have been calculated for the short latency responses (114, 113); and between 0.9-1.0 m/sec for the long latency ones, (113, 305). There seems to be no relation between the latency and the nature of the PSP's (excitatory vrs inhibitory).

Extracellular recordings in conscious monkeys, report similar short and long latency inhibitions (107). Conduction velocities falling in the same range, have been reported from rats, (88), although the monosynaptic nature of the response was not corroborated.

The/

The cause of the excitatory and inhibitory responses of nigral cells has been attributed in most cases, as possible involvement of different synaptic transmitters, (88, 114, 108). However, the possible stimulus spread to cortico-fugal fibres has been discussed, (134), although the lack of movement in anaesthetized stimulated animals questions this possibility, (108).

Few experiments consider the difference between the pars compacta and the pars reticulata of the substantia nigra, (Section 1). One report, describes that from the substantia nigra pars compacta only 60% of the cells were inhibited by striatal stimulation, while 95% were inhibited in the pars reticulata in rats, (88).

Correlating the anatomical placement of the electrode with the activity of the cells, it has been observed in rats, that cells in the substantia nigra pars compacta, have firing rates of 3-10 spikes/sec (88, 300) and peak inter-spike-intervals, (ISI's) between 60-250 msec (300). On the contrary cells in the pars reticulata have firing rates of 15-20 spikes/sec and ISI's between 10-50 msec, (88, 300). This has been considered as "the signature of the cells" (300), and could be used as a preliminary guide for their identification.

Summarizing, it could be said that in agreement to the feedback hypothesis, mainly inhibitory responses are observed in the substantia nigra after caudate nucleus stimulation, although consistent excitations are also detected, (305, 88, 204); possibly due to the participation of different synaptic transmitters, (88, 114, 108). Fibres of different conduction velocities are considered to be the cause for short and long latency responses, believed to be monosynaptic, (113, 114, 305).

As seen in the case of caudate cell recordings, there is a need for careful/

careful identification of recorded neurons; perhaps the differences observed in the firing rate and ISI's in both areas of the substantia nigra, (88, 309) should be used more often to differentiate them.

3E ELECTROPHYSIOLOGICAL RESPONSES OF SUBSTANTIA NIGRA CELLS TO DRUGS.

As has been previously mentioned, afferent fibres from the striato-nigral pathway may contain GABA, (Section 2. A2) and Substance P (Section 2.D1.2); on the other hand, dendrites or axon collaterals of dopamine cells also seem to release dopamine into the substantia nigra (Section 2.2.D). Therefore, it is relevant from the feedback point of view to study the effects of these drugs and their agonists and antagonists on the activity of nigral cells, and relate them to the electrophysiological results mentioned on the previous section.

E1. GABA AND NIGRAL CELL ACTIVITY

As electrophysiological studies indicate there is an inhibitory input to the substantia nigra from the caudate nucleus in cats and monkeys, and the striatum of rats. Since GABA is believed to be the inhibitory transmitter in some synapses in the nervous system of these animals, (252) and it is found in the substantia nigra, most of it presumably coming from the striatum, (Section 2.A.2), it is logical to suggest that GABA may be involved in the inhibitory response observed after the electrical stimulation of this nucleus.

Recordings of spontaneously active neurons in the substantia nigra are inhibited by the iontophoresis of GABA in this region in rats, (76, 230, 295) and the effect is reversed by picrotoxin either iontophorized (76) or injected intravenously in cats, (241). The effect of GABA is also/

also abolished by iontophoretic application of bicuculline, another GABA antagonist, (230). The positive field potential which accompanies the inhibition of the firing rate, is also reduced after the intravenous injection of picrotoxin, (241).

E2. SUBSTANCE P AND NIGRAL CELL ACTIVITY.

The depolarizations and action potentials also observed after caudate nucleus stimulation could be due to the release of substance P found in the striato-nigral pathway (Section 2, D.1.2). It has been shown that when iontophorized, it depolarizes cells in the ventral horn of the spinal cord (175) and cortical pyramidal cells (237) of rats, and in the cuneate nucleus of cats (180), suggesting that it could possibly act as an excitatory transmitter in the central nervous system.

Accordingly, after the iontophoretic injection of Substance P in the cells of the substantia nigra pars compacta of rats, there is an increase in the firing rate, (295, 81).

Even though the excitation produced after the application of substance P seems to be strong, it has been reported to be slow in its onset, taking from 7 to 30 sec to produce its effect, (180, 81, 237). Therefore, there is an argument about whether it is a "normal" synaptic transmitter capable of mediating transmission quickly (in terms of milliseconds) or on the contrary, influencing neuronal excitability over long periods, (180). Consequently, although it seems to act by depolarizing cell bodies, the function of substance P in general does not seem to be clearly understood.

E3. Dopamine and Nigral cell activity./

E3. DOPAMINE AND NIGRAL CELL ACTIVITY.

E3.1 AMPHETAMINE.

This drug appears to facilitate catecholamine action by promoting release and blocking reuptake at presynaptic terminals, (292, 27).

In the periphery, amphetamine seems to act by releasing noradrenaline from its terminals, although it has itself little or no direct effect on adrenergic receptors, (50). It seems to act similarly in the brain by releasing noradrenaline and dopamine from nerve terminals, (209). This is supported by the finding that pretreatment with a drug that inhibits catecholamine biosynthesis, alpha-methyl-para-tyrosine (AMPT) prevents the amphetamine stimulation of behaviour (e.g. increased locomotor and stereotyped behaviour, 9).

Furthermore amphetamine produces an increase in the accumulation of the O-methylated products of dopamine and noradrenaline (normetanephrine and methoxytyramine) after inhibition of monoamine oxidase, indicating an increase in the release of catecholamines (9). Perfusion of dextro-amphetamine through a push-pull cannula in the caudate nucleus of cats, results in a significant increase in the concentration of dopamine released (267, 202). The uptake of radioactive dopamine is significantly reduced in the rat striatum after dextro or levoamphetamine (71). In rats injected intraperitoneally with amphetamine 90 min before their sacrifice, the synthesis of dopamine is also reduced as indicated by the levels of radioactive water and dopamine in striatal slices incubated with radioactive tyrosine, (27).

Although it seems clear that the effect of amphetamine is presynaptic releasing catecholamines from their terminals, there is also evidence suggesting/

suggesting that it might act postsynaptically directly on catecholamine receptors, (106). Recording of cells in the caudate nucleus of cats after injection of 6-OHDA decreased their firing rate as in control animals, after amphetamine administration suggesting a postsynaptic effect since the catcholamine terminals were destroyed by the injection of 6-OHDA. However, since the dopamine content in this nucleus was only reduced by 42-87%, remaining undamaged terminals could be the responsible for the observed effect.

E3.2 EFFECT OF DOPAMINE AGONISTS, ANTAGONISTS AND RELEASING AGENTS.

The simplistic idea that a neuronal cell body and dendrites only receive the messages transmitted by other neurons, has become more complicated after what perhaps started as a control experiment looking at the effect of these agents in the substantia nigra.

Intravenous injection of apomorphine, decreased the firing rate of cells in the zona compacta of the substantia nigra and this was reversed by the systemic administration of haloperidol or chloropromazine in anaesthetized or unanaesthetized paralysed rats (2, 39, 1, 44).

L-Dopa which presumably is converted into dopamine thereby increasing the amount of dopamine potentially available, also depresses the firing rate of these cells (39).

Injections of 6-OHDA into the bundle of fibres leaving the substantia nigra, which presumably destroys dopamine cell bodies in this area, leaving other possible dopamine interneurons intact, not only reduces the fluorescence in this area homogeneously, but produces a selective loss of single units which had the firing pattern and responses to drugs characteristic of these cells, (1); confirming that/

that the cells in the substantia nigra zona compacta were the ones responding to the drugs. These results lead to the conclusion that the neurons which were producing dopamine as their transmitter also responded to it, suggesting they had dopamine sensitive receptors in their cell bodies, (1), which were later termed "autoreceptors", (52), (Section 2, 2.D).

Amphetamine injected intraveously also reduced the firing rate in the zona compacta of the substantia nigra and the effect was prevented or abolished by the administration of antipsychotic drugs, (39, 40, 41). Pretreatment with AMPT, abolishes the effect of amphetamine (39, 132), suggesting that in fact it decreases the firing rate by releasing dopamine. This evidence was considered to give great support to the feedback hypothesis; it was concluded that amphetamine was facilitating the release of dopamine in the terminals from nigral cells in the striatum and that it was interacting with receptors on postsynaptic cells which in turn sent their axons to the substantia nigra producing the observed inhibition, (Fig. 4).

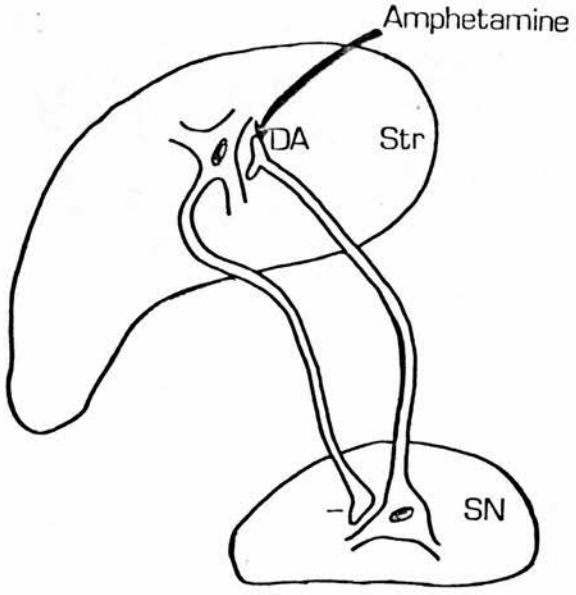

Fig.4 HYPOTHETICAL REPRESENTATION OF THE FEEDBACK PATHWAY, (132)

See Sections E3.2 and 3.F. Symbols as Fig. 1.

3F. IS THE ELECTROPHYSIOLOGICAL EVIDENCE CONSISTENT WITH THE FEEDBACK HYPOTHESIS?

By 1973, all the electrophysiological results seem to fit into this hypothesis (39, 52). The electrophysiology of the striatum was not taken much into account, but because of its contradictory results, it did not produce any conclusive evidence against this idea.

Further support was given once more by Bunney and Aghajanian in the same years, (40). They transected the brain in front of the substantia/

substantia nigra with a retractable knife, immediately before the recording. This reduced the effect of the intravenous injection of amphetamine causing "only a slight decrease in the activity below baseline rate", even at doses 8 times higher than usual, (40).

Moreover, iontophoretic injection of amphetamine into the zona compacta of the substantia nigra of normal rats, caused "a minimal slowing" of the firing rate of these cells, (40). It was concluded that the striato-nigral pathway was needed for the effect of amphetamine to take place, (Fig. 4).

Two years later, in 1975, what had seemed a coherent explanation was questioned by another group of researchers, (Groves et al, 129). Local injection of amphetamine through a cannula, placed in the striatum of rats, was reported to speed up cells in the substantia nigra pars compacta and reticulata, while the opposite effects were observed with the infusion of amphetamine in the zona compacta of the substantia nigra; cells in this area slowed down and simultaneous recordings indicated that cells in the striatum increased their firing rate. Since by this time it had been shown that dendrites, cell bodies, and/or axon collaterals in the substantia nigra release dopamine into this area, (Section 2, 2.A), it was concluded that amphetamine had a local effect on substantia nigra cells, so that the release of dopamine in this area produced their auto-inhibition, which in turn facilitated the discharge of striatal cells (disinhibition), increasing the firing rate in the striato-nigral pathway reinforcing the inhibition of the substantia nigra pars compacta and also inhibiting cells in the zona reticulata, (129), (Fig. 5).

It was also reported that pretreatment with AMPT (intraperitoneally) reduced/

reduced or abolished the depression of the neuronal firing rates of substantia nigra cells, by local infusion of amphetamine, (129, 130 - rats).

In an attempt to destroy the striato-nigral pathway, electrolytic lesions were done during the recording of nigral cells; these lesions included the crus cerebri and "the anterior border of the substantia nigra, invading both pars compacta and pars reticulata", (130), in these cases the local infusion of amphetamine did not have the usual depressant effect. It was concluded that somehow the lesion interrupted the release of dopamine in the substantia nigra, (130).

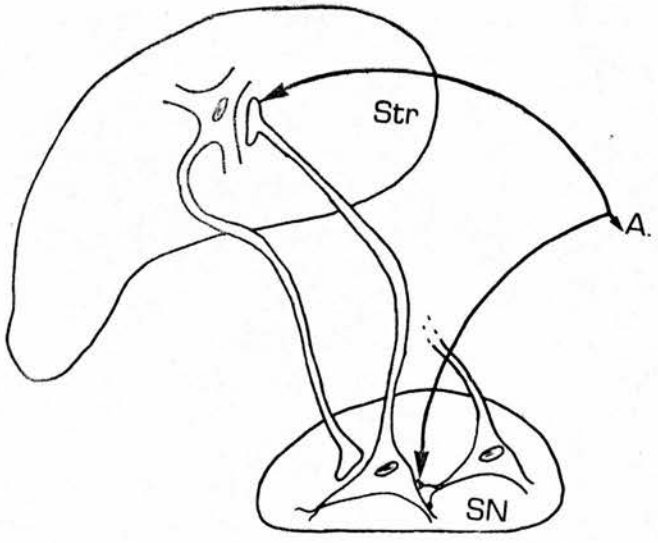

Fig.5 HYPOTHETICAL REPRESENTATION OF THE FEEDBACK PATHWAY (129).

See Section 3.3. A = amphetamine site of action.

An answer to the first report of Groves et al (129), came a year later by Bunney and Aghajanian (42), indicating that local anaesthetic effects could not be ruled out with an infusion of amphetamine into the substantia nigra, even although Groves et al (129) had emphasized that local anaesthetic effects were not observed, since the amplitude of the spikes remained the same. It was also reported this time, that lesions in the crus cerebri or in the tail of the striatum, immediately before the intravenous injection of amphetamine usually "attenuated" the effect although some cells had a "partial depression" in the firing rate even at low doses (0.8 - 1.6 mg/kg). It was also reported that chronic lesions in the crus cerebri, instead of the acute lesions they always performed, in some cases "return the ability of amphetamine to depress" these cells. Since the effect of amphetamine in some occasions was partial, an incomplete lesion or an action on dopaminergic autoreceptors was given as an explanation.

Last year, the two points of view were finally united by Aghajanian and Bunney (2) with what can be considered a good conclusion from the electrophysiological evidence available so far: "... It is evident both from the biochemical and physiological studies, that feedback mechanisms involved in the regulation of dopamine neurons are far more complex than originally supposed....The apparent existance of presynaptic dopamine receptors and their possible role in local feedback regulation must now be considered along with feedback mechanisms involving neuronal circuits and modulation of impulse flow...." (2, pag. 6).

4. DOES THE FEEDBACK PATHWAY EXIST?

After reviewing the literature about the feedback pathway, if a person is not thoroughly confused, it is probably because he does not know much about it. There are so many controversial reports that one begins to think that some must be reporting artefacts. As the techniques are improved and the ideas modified, so are the results of the experiments.

When the hypothesis of the feedback was first proposed, evidence to support it rapidly appeared. The anatomy indicated that the two pathways existed (Section 1), the increase in dopamine turnover after antipsychotic drugs was verified by different techniques and opposite effects were observed after dopamine agonists, (Section, 2.A). Also, antipsychotic drugs and dopamine agonists were found to increase and decrease the firing rate of dopamine cells respectively, (Section 3.E). All giving strength to the feedback hypothesis. When it appeared convincing that there was a feedback control of dopamine metabolism, reports indicating that another kind of control also existed started to emerge, and the results began to look confusing.

For instance, using anterograde and retrograde neuroanatomical tracing techniques it was confirmed that the striato-nigral pathway did not end directly onto dopamine cell bodies, (Section 1.A), however, this did not upset the idea of a feedback as much as the biochemical results. In vitro studies showing that antipsychotic drugs have the same effects as invivo, and the paradogic increase in dopamine after a lesion of the nigro-striatal pathway (Section 2.B.2.1) are perhaps the strongest evidence against the feedback, since the hypothesis predicted opposite effects.

Electrophysiological results complicated the picture as well.

Dopamine/

Dopamine cells were inhibited by direct application of dopamine or its agonists, (Section 2.D; 3.C.1), and release of dopamine within the substantia nigra was demonstrated, (Section 2.D) indicating that antipsychotic drugs could also act presynaptically.

It has also been shown that other areas of the brain (e.g. locus coerule us, hippocampus) respond in a similar way to transmitter agonists or blockade of impulse flow, even though no anatomical evidence exists of a feedback loop of the kind postulated for the dopamine system, (256, 34).

As controversial evidence was gathering also alternative explanations to the feedback were offered, for instance, it was proposed that the regulation of dopamine metabolism takes place in the presynaptic terminal mediated by presynaptic receptors and the consequences of depolarization such as the influx of calcium across the membrane (Section 2.B.2.3). Local circuits within the striatum (Section 2.C) or the substantia nigra, (Section 2.D) have been also proposed as regulators of dopamine metabolism. However, none of the alternative hypothesis proposed so far, can explain all the controversial results by themselves, and the experiments which support them are not always consistent (Section 2.E; 3.F).

Perhaps because the idea of a feedback control was so clear when it was first proposed, it is very difficult to see that it may not in fact exist, and the evidence against it has found opposition. Another reason for not abandoning the idea of a feedback loop could be that the alternative hypothesis is not that convincing. However, since the feedback hypothesis has lost strength, even its most tenacious defendants/

defendants are now proposing that besides the feedback there may be another way of control of dopamine synthesis (52, 2).

The conservative point of view is now prevailing, it is considered that the feedback hypothesis still can be supported but for those cases where the hypothesis does not predict the results, other explanations are elaborated ad hoc.

It has been shown that the striatum and the substantia nigra can regulate their synthesis of dopamine if left without any communication from each other. This may be related to the "recovery of function" commonly observed in the nervous system, (183). In other words, an animal even with an extensive destruction of its brain can recover skills temporarily lost after the lesion, perhaps because the brain has more than one system like the dopamine one which can regulate itself even though its connections are interrupted.

CHAPTER II

BIOCHEMICAL ANALYSIS OF THE STRIATUM AND THE SUBSTANTIA NIGRA AFTER A LESION IN THE STRIATO-NIGRAL PATHWAY.

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A. INTRODUCTION.

The feedback hypothesis could not have existed if the anatomy had not confirmed that there were fibres interconnecting the striatum with the substantia nigra, (Chapter I, Section 1). Since the striato-nigral and the nigro-striatal pathways run close together, it seemed very difficult to make a lesion in one pathway without damaging the other. Early attempts to demonstrate that this interconnection was responsible for the control of dopamine synthesis, involved a hemisection of the brain between the striatum and the substantia nigra, (200), which would certainly damage both pathways.

The development of techniques involving transport of material anterogradely or retrogradely in the neurons, has helped to trace both pathways (Chapter I, 1.A). Studies of the anatomy of the striato-nigral pathway in this way done in our laboratory by Tulloch et al (282) have confirmed that it is wholly contained within the crus cerebri at the level of the hypothalamus, well separated from the nigro-striatal pathway. It occurred to us that placing a small lesion in the crus cerebri at this level, offered the possibility of opening the so called feedback loop and in this way evaluating the action of the striato-nigral pathway in the control of dopamine metabolism.

It has been shown that hemisections (200) or lesions in the striatum or globus pallidum (169, 200) produced a decrease in Gama-amino-butyric-acid (GABA) in the substantia nigra and also that Glutamic acid decarboxilase/

decarboxylase (GAD) activity is reduced in this area after lesions in the crus cerebri (117, 236), (Chapter I, Section D.1). In the experiments reported here it was decided to measure GAD and GABA in order to determine if these fibres contain GABA. Since during the course of this experiment, the concentration of Substance P (SP) was reported to decrease in the substantia nigra after lesions in the striatum and globus pallidum (117, 236) we decided to measure its concentration after lesions of the fibres running in the crus cerebri.

It has been reported that the afferent fibres to the substantia nigra coming from the striatum run close together with the afferent fibres from the globus pallidum (Chapter I, Section, 1.A). Therefore a lesion of the afferent pathway will damage both fibre systems and will not differentiate between the effects of each structure. Kainic
i, a neurotoxic which has been shown to damage cell bodies
le sparing afferent terminals and axons of passage,(262),was
ected into the globus pallidum in order to find out if that structure
ne was responsible for the decrease observed in GAD activity.In this case
the fibres of the striato-nigral pathway, should be left intact while the cells giving rise to the pallido-nigral pathway are damaged.

The results reported in this Chapter suggest that these lesions in the crus cerebri do in fact spare the dopamine containing fibres of the nigro-striatal pathway and deplete the substantia nigra of GABA and SP. No effect on dopamine metabolism in the nigro-striatal dopamine neurons was observed, supporting the idea that the striato-nigral pathway is not part of a feedback loop.

B. PROCEDURE.

The/

The animals were lesioned in the crus cerebri, allowed to recover for a week, tested for turning behaviour and sacrificed at least a week later. The tissue was dissected and the biochemical determinations were done. Then the groups were selected according to A) the extent and placement of the lesions indicated by the histological analysis, or B) the drugs administered to the animals before their sacrifice. In the pharmacological experiments the histological analysis was done for each animal in the group and the extent of the lesion was kept constant by discarding those rats with incorrect lesions.

The mean number of the concentration of a particular chemical or the activity of an enzyme for the control and experimental conditions were plotted and statistical significance of the difference estimated.

C. METHODS.

C1. STEREOTAXIC SURGERY.

Male albino wistar rats which weighed between 190-210g at operation were used. The animals were initially anaesthetized by circulating an air/fluothane mixture through a plastic box. Once the flexor reflexes were lost, the rat was transferred to a David Kopf stereotaxic frame where the anaesthetic was administered through a mask fitted over the nose and mouth. The rat was maintained at 37'C by warming under the abdomen with an homeothermic blanket (24, A. Epil). The skull was exposed by a sagital incision in the skin, the overlying periosteum was scraped away and a 3 mm hole was burred with an electric drill (Renda, England). The tooth bar of the frame was fixed at -2.5mm below the ear bar zero.

C1.1 ELECTROLYTIC LESIONS.

A bipolar electrode made of twisted teflon-coated Tungsten wire (Phoenix Wire Co.) 0.16 mm in diameter, insulated except for the tip, was lowered into the brain at the following co-ordinates, aiming for the ventro-medial crus-cerebri at the level of the hypothalamus.

According to the Koning and Klippel atlas of the rat brain, (174):

Antero-posterior: +3.7

Lateral: -2.4

Vertical: -7.8 Dura Membrane as reference point

A current provided by a 6v battery measured with an ammeter connected in series, passed through the electrode for a period of time such that an electrolytic lesion was always made with a total charge of 6m Coulombs, independently of changes in resistance.

Afterwards the electrode was removed, the surface of the skull sprayed with an antibiotic powder (Polybactrin, Calmin Ltd.), and the skin sutured with cotton thread.

For lesions involving the whole crus cerebri, the electrode was inserted at an angle of 46' at the following co-ordinates, (174), with the same reference points as above:

Antero-posterior: +3.7

Lateral: -7.7

Vertical: -6.5 and -7.5 Dura membrane as reference point.

In order to make a bigger lesion, current passed at two different heights 1mm appart, 6m Coulombs being delivered each time.

C1.2/

C1.2 KAINIC ACID INJECTIONS.

Kainic Acid (Sigma 5mg/ml) was dissolved in saline 0.9% (w/v) and the pH adjusted to 7.4. A needle for cartridge syringes, gauge 30, 0.46mm diameter, was lowered into the brain at the following co-ordinates (174), aiming for the globus pallidum.

Antero-posterior:	+6.0
Lateral:	-3.6
Vertical:	-5.6

The volume injected was 0.1 µl (0.5µg) over a period of 2 min. The needle was left in place for further 5 min after the injection to allow diffusion to take place.

C2 POST-OPERATIVE OBSERVATIONS.

The animals were allowed to recover for a week. During this period attention was payed to any loss in weight and to the regular ingestion of food and water; if necessary, a special diet of milk and bread was provided.

After a week, their ability to display turning behaviour (Chapter IV Section C1.1) was tested after an intraperitoneal injection of apomorphine hydrochloride (Macfarland Smith Ltd., Edinburgh, 2 mg/kg).

Rats which turned 100 times or more in 30 min were considered as having a successful lesion. In this way they were selected for their different treatments, and the exact location of the lesion was verified afterwards by histological analysis (Chapter IV Section D1.).

C3. BIOCHEMICAL ANALYSIS.

C3.1/

C3.1 DISSECTION OF TISSUE.

At least one week after the apomorphine test, the rats were killed by cervical dislocation with a strong and rapid strike on the back. Immediately, the animals were decapitated and brains rapidly removed and placed in cold saline, (9% w/v).

The dissection was performed over ice. The brain was placed on a plate specially designed to hold a pair of blades approximately 1mm apart, (Fig. 6). A coronal section behind the mammilary bodies was cut with these blades. The piece of tissue was placed flat on a dissection plate and a rectangular piece containing the interpeduncular nucleus was dissected. The two substantia nigra nuclei were removed by withdrawing the overlying cortex and making an oblique cut at the level of the medial lem niscus on each side.

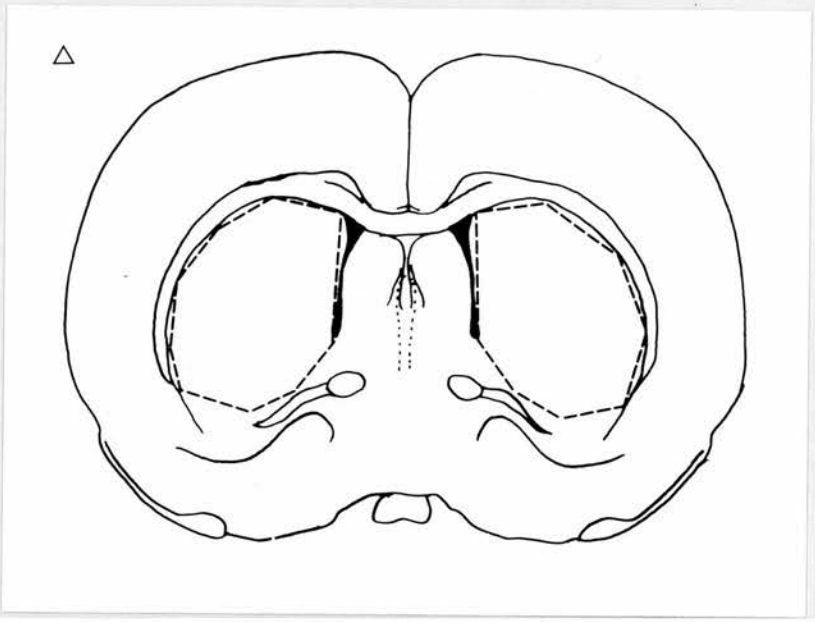
With the rest of the brain, a coronal section was made at the level of the opticchiasma. The posterior portion was kept for histology and the striatum was removed from the anterior part. First the nucleus accumbens was cut and then with curved forceps the striatum was taken out until the fibres of the corpus callosum were exposed. The frontal piece was also kept as control tissue.

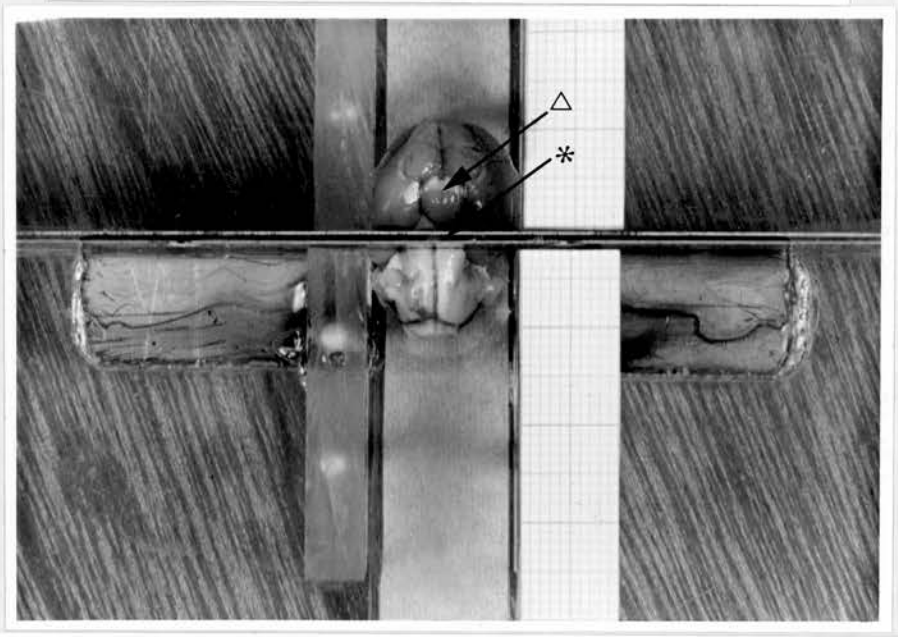
The pieces of tissue were wrapped in labelled aluminium foil and frozen in liquid nitrogen. The freezing procedure was randomized and the whole dissection did not take more than 5 minutes. The samples were kept in liquid nitrogen until required for biochemical assay.

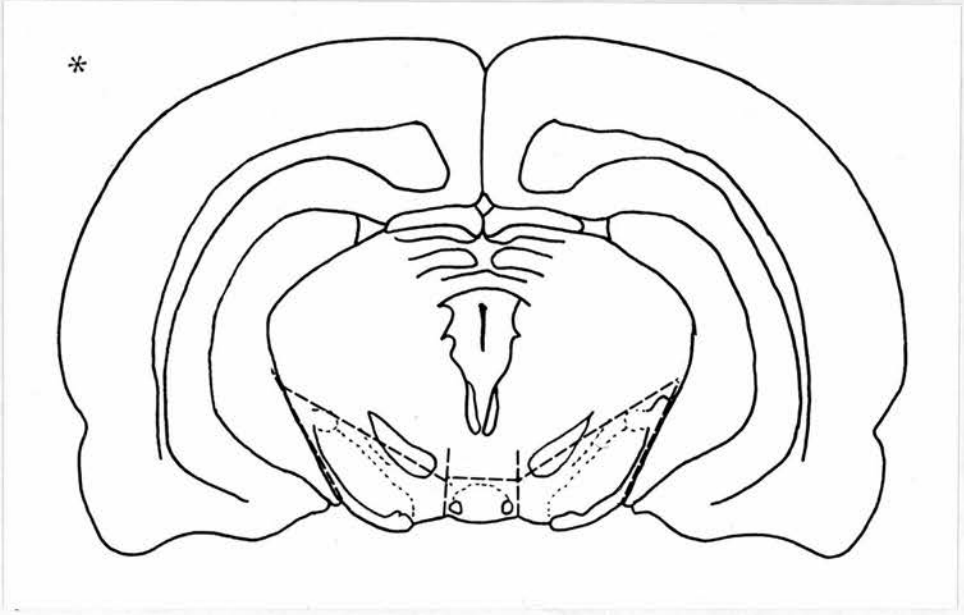
C3.2 DETERMINATION OF DOPAMINE CONCENTRATION.

The radiometric method described by Palkovits et al (231) was used. The procedure is based on the O-methylation of catecholamines by catechol-O-methyl transferase.

Tissues/







Tissues were homogenized in 0.1 N perchloric acid, (AnalaR, BDH Chemicals Ltd.), 300 µl/10mg of wet tissue for the striatum, and 300 µl/50mg of wet tissue, for the substantia nigra. This is to keep the homogenates between the range of detection of the assay, 5-100 µg dopamine/g tissue.

After centrifugation, 300 µl of the supernatant were transferred to 15ml glass stoppered centrifuge tubes in duplicate for each sample, on an ice bath.

Standards of 25ng of Dopamine in 10µl of distilled water were added to another set of tubes with 300µl of brain extract. Blanks were prepared by adding 300µl of perchloric acid to a third set of tubes.

The reaction was initiated by addition of 100µl of the incubation mixture containing the following components: 500 µg of dithiothreitol (Sigma); 0.5 µmoles of magnesium chloride; 140 µmoles of tris (hydroxymethyl) - methylamine buffer, pH.9.6; 1ml of catechol -O- methyltransferase (prepared from rat liver by the method of Coyle and Henry, 603), and 2.5 µCi of (^{3}H) methyl-S-adenosyl-methionine, (specific activity: 12.6 Ci/mM - Radiochemical Center Ltd).

The reaction mixture was incubated for 60 minutes at 37'C, and was stopped by addition of 500µl of 0.5 molar Borate Buffer, pH 10. After the addition of 50 µl of a non-radioactive carrier solution containing 7 µg of methoxytyramine; 3 µg of normetanephrine (Sigma); 3µg of metanephrine (Sigma) and 1g of ethylenediaminetetra (BDH Chemicals Ltd.); the O-methylated products were extracted into 9ml of water-saturated ethyl acetate-methanol (10:1, v/v), by shaking for 30 sec. The phases were separated by centrifugation (200 x g force) for 2 min. As much as possible of the organic phase was transferred to another tube. In this occasion/

occasion 500µl of 0.5 M boric buffer, pH 10, were added. After the tubes were shaken for 30 sec and centrifuged for 2 min., the organic phase was transferred to another set of tubes containing 500µl of 0.1N hydrochloric acid, into which the O-methylated products were extracted into the aqueous phase by shaking for 30 sec. The phases were separated by centrifugation and the organic phase aspirated and discarded. The acid phase was washed with additional 2ml of water-saturated ethyl-acetate; after mixture and centrifugation, the ethyl-acetate was aspirated off and discarded. To the remaining phase, 500 µl of 0.5 M sodium phosphate buffer, pH 7.5 were added and the tubes were transferred to an ice bath.

To separate normetanephrine from methoxytyramine the side chain of normethanephrine was adhered to the beta-hydroxyl position with 50µl of a freshly prepared 3% (w/v) sodium methaperiodate solution. Three minutes after the addition of this solution, 50µl of 10% (v/v) glycerol were added to stop the reaction. The (^{3}H)-methyl-vanillin derived from the cleavage of the normethanephrine was extracted into 10ml of toulene by mixing for 30 sec. After the tubes were centrifuged, 9.5ml of the organic phase can be transferred to another tube for the determination of noradrenaline, but since in this study only dopamine was taken into account, further details will not be included.

The aqueous phase left in the tubes after discarding the toulene was washed with another 5ml of toulene. After mixing and centrifuging, the organic phase was aspirated off and discarded, and 500µl of 1.0 M borate buffer, pH 11, and 6ml of toulene-isoamyl alcohol (3:2, v/v), were added to each tube. The (^{3}H)-methyl-methoxytyramine was extracted into the organic phase by mixing and centrifuging. 5ml of the/

the organic phase were transferred to a scintillation vials containing 10ml of NE-260 Scintillant, (Nuclear Enterprises Ltd., Edinburgh).

The samples were counted in a Liquid Scintillator, (Nuclear Chicago), for 1 min. The results were expressed in ug of dopamine/g of wet tissue.

C3.3 DETERMINATION OF 3,4-DIHYDROXYPHENYL-ACETIC ACID (DOPAC) AND HOMOVALLINIC ACID (HVA) CONCENTRATION

This assay is based on the formation of hexafluoro-iso propyl esters and their estimation by gas-liquid chromatography (GLC), combined with electron capture detection.

The method was described by Pearson and Sharman (234) and modified in our laboratory by Nicolau (214).

The samples were homogenized in 0.4 N Perchloric acid (BDH Chemicals Ltd., AnalaR), mixed for 45 sec and centrifuged at low speed for 4 min. The supernatant was transferred to an eppendorf tube (Eppendorf, Germany) containing approximately 0.5g of potassium chloride, with which the perchloric acid forms potassium perchlorate plus hydrochloric acid. The HVA and DOPAC will attach to the acid and remain in the supernatant. The samples were kept on an ice bath.

After shaking and centrifuging, the supernatant was transferred to another set of eppendof tubes containing 0.5 ml of toulene in order to eliminate the lipids. After shaking and centrifuging, the toulene was aspirated off and discarded. This procedure was done twice. In order to precipitate remaining proteins, the tubes were closed and left in liquid nitrogen for 10 min. After freezing the pressure inside was equalized by opening the cap for a moment, afterwards the tubes were/

were closed again and centrifuged for 4 min. The supernatant was then transferred to another set of tubes containing 0.5ml of ethyl acetate. After shaking and centrifuging, the supernatant was transferred to a set of glass vials containing 0.5ml of ethyl acetate. The samples were shaken and centrifuged for 4 min and left to dry completely under a stream of dry nitrogen at room temperature. This ethyl acetate extraction procedure was performed three times for each sample. In all instances the samples were evaporated to dryness under nitrogen.

To each glass vial, 200µl of trifluoroacetic anhydride (Aldrich Chemical Co. Ltd.), and 100µl 1,1,1,3,3,3,-Hexafluoropropan-2-ol (BDH Chemicals Ltd.), were added. This produces an ester with HVA and DOPAC which has a boiling point lower than these two products, and therefore can produce a vapour containing them. The fluoride ions provide more eletrons and increase the sensitivity of the electron capture detector in the GLC.

Each vial was closed tightly, mixed for 5 sec and left in a plate at 115'C (Dri-Block-Unit 3 Griffin and George Ltd.) for one hour. The reaction vials were allowed to cool to room temperature before opening and evaporating the contents just to dryness under a stream of dry nitrogen (until a brown residue is left). To this residue 1ml of ethyl acetate containing 0.1 µg of pentafluorophenyl benzoate (internal standard) was added. The contents were shaken for 30 sec and transferred to the vials used in the GLC.

The GLC fitted with a Nickel 63 electron capture detector, was maintained at 250'C. The carrier gas was argon containing 5% methane. It was delivered at a flow rate of 50ml/min which corresponded to a gas pressure of 40 pounds/sq.inch. The chromatograph column consisted of

2%/

2% SE liquid phase coated on chromosorb Q(Hewlett Packard)

The areas of the DOPAC and HVA peaks were measured in each case, and the ratio of metabolites peak area to internal standard peak area was calculated. These ratios were compared to those in a standard curve, and the amounts of HVA and DOPAC in each sample were calculated, and expressed as µg/g wet tissue.

C3.4 DETERMINATION OF GAMMA-AMINO-BUTYRIC-ACID (GABA)CONCENTRATION

This assay is based on the formation of a derivative thought to be 1,1,1,3,3,3 hexafluoro-isopropryl-N-trifluoroacetyl-4-aminobutyrate and its measurement by GLC and electron capture detection. The method was described by Pearson and Sharman (233).

The tissue was homogenized in 700µl of 0.1 N hydrochloric acid. Along with crystalline KCL, 25µl of 12 M perchloric acid were added to the homogenate. The sample was centrifuged at low speed for 5 min, then 25µl of the supernatant were transferred to another set of tubes containing 1ml of 1 M hydrochloric acid. After mixing, 0.5ml were allowed to flow under gravity through a chromatographic column prepared as follows.

The resin, amberlite CG 120 (100-200 mesh; BDH Chemicals Ltd.) was first cleaned by stirring in 2 M hydrochloric acid, washing several times with water. After repeating this procedure three times, the resin was poured to a depth of 2 cm into a Pasteur pipette fitted with a glass filter at one end to retain the resin. Prior to application of the homogenates to the column, 2ml of 2 N sodium hydroxide solution was passed through the resin followed by 3ml of water. Then 2ml of 1 M hydrochloric acid were passed through column/

column and the resin was finally washed with 10ml of distilled water.

Once the hydrochloric acid containing the tissue sample was allowed to flow through the column, it was washed with 10ml of water. GABA was removed from the resin with 2ml of 2 M NH_4OH solution and collected directly into a reaction vial.

The sample was then evaporated to dryness in a vacuum desicator over phosphorus pentoxide. The dry residue was dissolved in 0.2ml of trifluoroacetic anhydrid and 0.1ml of hexafluoroisopropanol were added. The reaction vial was tightly closed and left for 1 h at room temperature. The contents of the vials were then evaporated just to dryness under a stream of dry nitrogen at room temperature. To this residue, 1ml of ethyl acetate was added and the vials were closed and shaken, before placing them into the GLC.

The conditions of the GLC were the same as those described for the estimation of HVA and DOPAC (Section B3.). For each run of samples, a standard curve for GABA was prepared, using the peak heights derived from the known amounts of GABA. The results were expressed in ug/g wet tissue.

C3.5 DETERMINATION OF GLUTAMIC ACID DECARBOXYLASE (GAD) ACTIVITY.

The activity of GAD enzyme was calculated by the combination of the radiochemical assay methods of Urquart et al (289) and Drummond and Phillips (90). The method involves the measurement of the amount of radioactive carbon dioxide evolved from L-(1-^{14}C)-glutamic acid on incubation with a tissue homogenate in the presence of pyridoxal phosphate, an essential cofactor of the enzyme. Labelled carbon dioxide is trapped in protozol and measured by scintillation counting.

The/

The tissue was homogenized with a homogenizing buffer (10 µl/mg tissue) containing the following: 10µl of phosphate buffer, pH 6.5; 100µl of 10 M pyridoxal-5-phosphate (Sigma; to form glutamic acid); 10µl of Triton-X-100, (to emulsify the sample), and 10ml of distilled water.

To another set of tubes, 10µl of the homogenate were transferred. The incubation mixture contained (final concentration), 100 mM potassium phosphate buffer, pH6.5; 0.5 mM pyridoxal-5-phosphate; 0.5 mM dithiothreitol; 1.22 mM L-(1-^{14}C)-glutamic acid, (specific activity 5.5 mCi/mM) diluted with 0.5 mM unlabelled glutamic acid; 0.1 mM Triton-X-100; 1 mM sodium arcenite anddistilled water, 10% (v/v).

Blanks were prepared by mixing 10µl of the homogenizing buffer with 10µl of the incubation mixture. To each tube containing the tissue homogenate, 10µl of the incubation mixture were added, and the tubes placed into a scintillation vial containing 250µl of protozol, (to trap the ^{14}C carbon dioxide liberated in the reaction). A rubber seal was used to close the scintillation vial. After incubation for 20 min in a warm bath of 37'C, the reaction was terminated by the addition of 0.2ml of 6 N Sulphuric acid injected through the rubber seal into each eppendorf tube. The vials were left in the warm bath for one hour in order to collect into the protozolthe ^{14}C carbon dioxide released. The outside walls of the eppendorf tube were then rinsed with 2ml of absolute alcohol on their removal from the scintillation vial. 10ml of toulene scintillant were added to the protozol and the alcohol remaining in the vials. After a minimum of half an hour to eliminate luminescence produced by the toulene, the samples/

samples were placed in the scintillation counter (Nuclear Chicago), and the radioactivity measured for 1 min. The number of counts per min for each sample after subtraction of the blank value, were corrected, for the counting efficiency, the time of incubation, the amount of protein present in every individual portion of the homogenate used, in every individual assay, and the specific activity of the radioactive glutamate in the assay.

To estimate the contents of protein in the sample, 5µl of the tissue homogenate were treated with the method of Lowry et al (193). The results were expressed in nmoles of $^{14}CO_2$ formed per mg of protein in the sample per hour of incubation.

C3.6 ESTIMATION OF SUBSTANCE P (SP) CONCENTRATION.

The method was described by Kanazawa and Jessell (159). SP was first extracted by a modified version of the procedure described by Chang and Leeman (59) with aceton in hydrochloric acid. The SP obtained in this way was measured by radioimmunoassay using the method described by Powell et al (239). Antibodies to SP formed in rabbits and guinea pigs were incubated with the samples to be assayed for 24 hr; radioactively labelled antigen, 125-Iodine labelled SP, was added and the incubation continued for another 48 hr. The antibody-bound label was separated from label free in the incubate, by differential absorbtion onto charcoal and the radioactivity determined. Owing to competition for the limited number of antigen-binding sites available, the amount of labelled SP-bound antibody drops as the concentration of the unlabelled antigen increases. The results obtained were compared with standard curves obtained with samples of known content of SP. The results/

results were expressed in ng/g wet tissue.

C4. HISTOLOGICAL ANALYSIS

The tissue left for histological analysis was stored in a deep freezer on a glass beaker containing ice, covered with cling film, (C.E. Payne & Sons Ltd.)

Frozen sections 20-40µm thick were cut with a Cryostat (Linde, Germany) kept at -40'C. The sections were melted onto a glass microscope slide (76 x 26mm, Chance Proper Ltd.), for further staining.

C4.1 STAINING OF TISSUE.

The method used was a modified version of the Kluver and Barrera technique (173) for staining myelinated fibres and cell bodies.

The tissue was dehydrated in 70% alcohol followed by 95% alcohol both for 5 min. The slides were transferred and left for 10 min in a 95% alcohol solution containing 1 mg/ml of Luxol Fast Blue (BDH Chemicals Ltd.), and 5µl/ml of 10% solution of acetic acid. Then the slides were rinsed in distilled water, immersed into 0.05% (w/v) lithium carbonate for 3 min and then placed in 70% alcohol for another 3 min. This procedure was repeated until clearly differentiated staining of myelinated tracts were observed. The slides were transferred to a solution containing 0.1% (w/v) cresyl fast violet (Fluka, A.G., Buchs, S.G., Switzerland) in distilled water, and left for 5 min. Afterwards they were rinsed and dehydrated in 95% alcohol for 5 min and in absolute alcohol for 10 min and cleared in Xylene (Analar) for 10-15 min. Canada Balsam in Xylene (BDH Chemicals Ltd.) was used as a mounting media. The slides were studied under a light microscope.

C5/

C5 DRUGS ADMINISTERED.

	DOSE mg/Kg	CONCENTRATION mg/ml	TIME OF INJECTION BEFORE SACRIFICE min
Apomorphine Hydrochloride (Macfarlan Smith Ltd.)	2.0	2.0	30
Haloperidol (Serenace, Searle & CO.)	1.0	1.0	30
Alpha-methyl-para-tyrosine (Methyl-ester, Sigma)	200.0	100.0	90
Alpha-methyl-para-tyrosine	200.0	100.0	90
plus			
Haloperidol	1.0	1.0	30

All these drugs were administered intraperitoneally.

C6 STATISTICS.

The statistical significance of the mean values for two different groups was estimated with the student's t-test for small samples for equal or unequal variances according to the case, (26).

The t-test for paired observations (142) was used if a group was compared against itself, that is, the same group before and after an experimental treatment or the comparison of values obtained on one side of the brain against the other.

Since a direction in the difference was expected (e.g. a decreased amount of a possible transmitter), a one tailed rejection region was used to test the significance of the difference.

The null hypothesis was rejected using a significant level(P) not greater/

greater than 0.05.

In the tables, the mean number was represented as $\bar{X}$; the standard deviation as σ and the total number of observations as N.

The percentage decrease was calculated as follows: $100 - \frac{L \times 100}{C}$ where L is the value obtained for the lesioned side, and C is the amount obtained for the unlesioned side or the control group, specified in every case.

Fig.7 Electrolytic lesion (dotted line) in the VENTRO-MEDIAL AREA of the crus cerebri. Staining: Kluver and Barrera technique, (Section B4). Calibration mark: 100µm.

D. RESULTS.

D1. THE ACTIVITY OF GAD AND THE CONCENTRATION OF SP AND GABA IN THE SUBSTANTIA NIGRA.

A lesion in the ventro-medial portion of the crus cerebri typical of the rats studied under this section is shown in Fig. 7.

A group of 10 lesioned rats showed a significant decrease in GAD activity in the substantia nigra when compared against the substantia nigra values of the unlesioned side of the brain or the GAD activity in this area for a control group. A similar reduction in GAD activity after the lesion in the crus cerebri, was also observed in the interpeduncular nucleus, (Table I).

The concentration of GABA in the substantia nigra determined in 3 rats also showed a significant decrease in the lesioned side compared with the unlesioned side or against the control group, (Table I).

Twelve lesioned rats on which the concentration of SP was determined in the substantia nigra showed a significant decrease on the lesioned side. This reduction did not produce any significant alteration in the concentration of dopamine in the striatal and frontal areas, (Table II). The results presented in this section are summarized in Fig. 8.

D1.1 POST-OPERATIVE OBSERVATIONS.

After the electrolytic lesions the rats did not lose weight during the first week after the operation and did not show any gross alteration in its motor performance. As discussed in Chapter IV, these rats displayed turning behaviour towards the lesioned side if injected intraperitonealy with apomorphine, (Fig. 8).

TABLE I

GAD ACTIVITY AND THE CONCENTRATION OF GABA

		SUBSTANTIA NIGRA				INTER-PEDUNCULAR NUCLEUS	
		GAD		GABA		GAD	SIGNIFICANCE P<
		L	C	L	C		
EXPERIMENTAL GROUP	$\bar{X}$	239.0	480.1	98.8	183.1	159.4	0.001 ———
	σ	92.0	98.3	31.6	28.2	41.8	0.01 -------
	N	10		3		10	
CONTROL GROUP	$\bar{X}$	576.9		287.3		251.1	L Lesioned side
	σ	112.3		141.7		7.3	C Unlesioned side
	N	4		8		2	
PERCENTAGE DECREASE CONTROL GROUP 100%		58.6		65.5		36.5	
UNSUCCESS-FUL LESIONS	X	398.8	460.1	118.3	199.8		
	σ	129.8	89.3	49.9	21.2		
	N	4		4			

able 1. Mean values ($\bar{X}$) of GAD activity and GABA concentrations in substantia nigra and interpeduncular nucleus of Crus cerebri lesioned rats, (experimental group), control group and unsuccessfully lesioned animals. For this and the following tables:

A. The percentage decrease was taken using the control value as 100% (Section C6)

B. The lines indicate where the differences in the values are statistically significant.

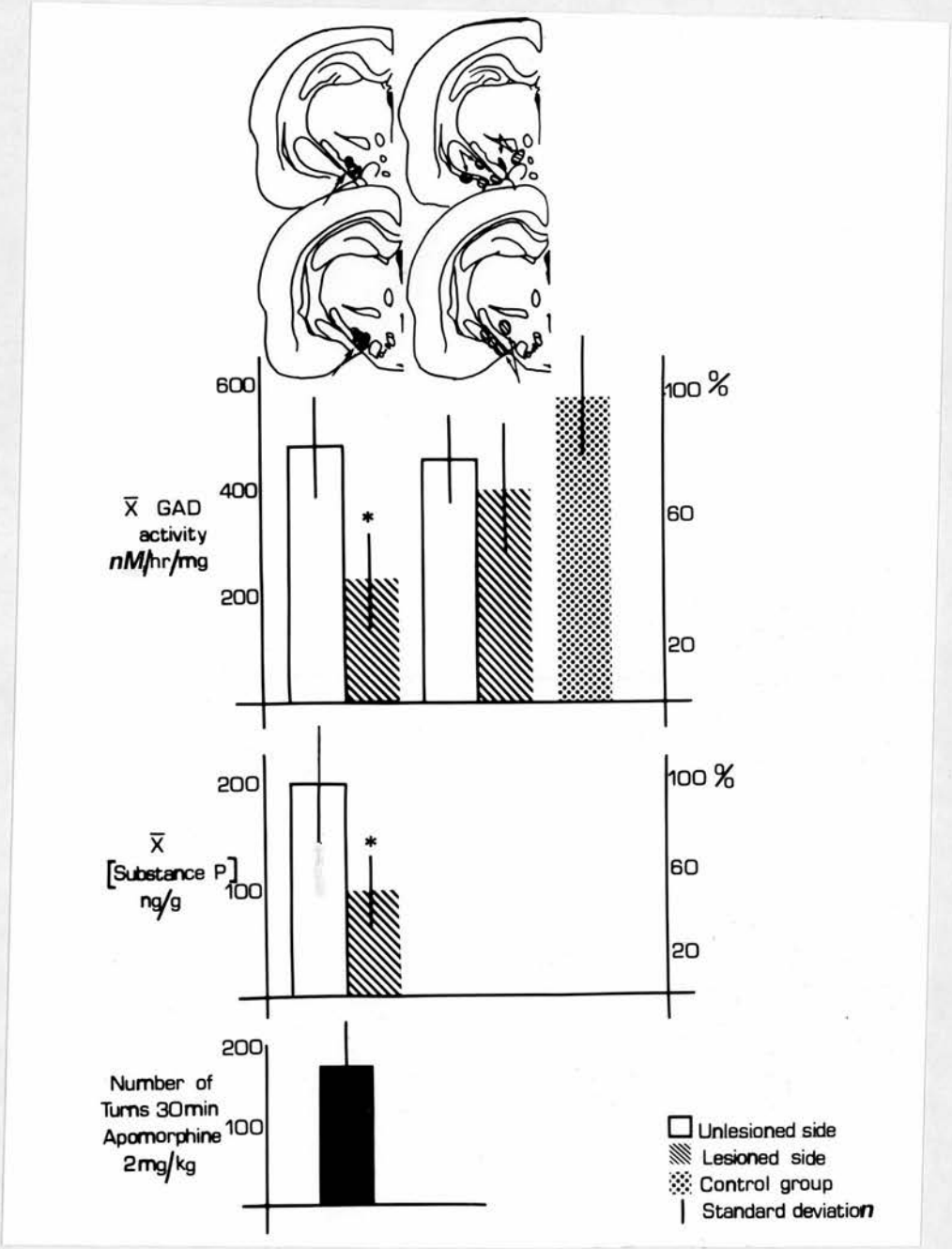


Fig. 8 GAD activity and the concentration of SPin the SUBSTANTIA NIGRA for lesions plotted on diagrams reproduced from two levels from the Konig and Klippel atlas of the rat brain, (174). Lower graph: behavioural observations one week after the lesion. *Difference statistically significant.

TABLE II

		Lesioned side	Control side	% Decrease	Significance P<
SUBSTANCE P	$\bar{X}$	94.06	194.64	51.68	0.005
	σ	46.86	107.16		
	N	12			
DA STRIATUM	$\bar{X}$	9.27	9.59	3.3	N.S.
	σ	1.50	1.70		
	N	12			
DA FRONTAL	$\bar{X}$	0.68	0.84	19.0	N.S.
	σ	0.20	0.28		
	N	12			
NUMBER OF TURNS/30 MIN	$\bar{X}$	163.9			
	σ	74.6			
	N	12			

D1.2 CONTROL GROUPS.

Animals with lesions in other areas around the crus cerebri (unsuccessful lesions), like the hippocampus, amygdala, zon· incerta and Forel's fields, did not show a significant decrease in GAD activity or in the concentration of GABA in the substantia nigra, compared against themselves or against a control group of unlesioned rats, (Table I).

Fig. 9 Electrolytic lesion (dotted line) in the LATERAL PORTION of the crus cerebri. Staining: Kluver and Barrera technique (Section B4.). Calibration mark: 100µm.

D2. EXTENT OF THE LESION AND GAD ACTIVITY IN THE SUBSTANTIA NIGRA.

Attempts to make a lesion in the crus cerebri in different places in order to relate the extent of the lesion with the decrease in GAD activity in the substantia nigra, produced a group of 10 rats, 3 with lateral lesions, (Fig. 9), 3 with medio-ventral lesions (Fig. 7) and 4 with all the crus cerebri destroyed.

TABLE III

EXTENT OF THE LESION AND GAD ACTIVITY IN THE SUBSTANTIA NIGRA.

		LATERAL		MEDIAL		COMPLETE		CONTROL GROUP
		L	C	L	C	L	C	
GAD ACTIVITY	$\bar{X}$	349.9	488.8	227.7	508.6	242.7	488.9	576.9
	σ	17.5	51.6	82.6	72.2	93.7	122.3	112.3
	N	3		3		4		4
% DECREASE:								
UNLESIONED SIDE A 100%	%	28.4		55.2		50.3		
CONTROL GROUP AS 100%	%	39.3		60.5		57.9		
SIGNIFICANCE P <		0.01		0.005		0.005		0.05 (against each group)
NUMBER OF TURNS/ 30 MIN	$\bar{X}$	83.33		163.0		191.6		
	σ	48.7		40.3		31.9		
	N	3		3		4		

L Lesioned side

C Unlesioned side

All lesions produced a significant decrease in GAD activity in the substantia nigra of the lesioned side, compared against the unlesioned side or against the control group. The animals with lesions in the medio-ventral portion of the crus cerebri showed the highest decrease (60.5%) in GAD activity in the substantia nigra, while the rats with lesions in the lateral portions showed the smallest decrease (39.3%) compared against the control group, (Table III).

The animals which produced the biggest mean number of turns (191.6) were the ones that had the largest lesion, and the rats with lesions in the lateral portion of the crus cerebri had the smallest mean number of turns (83.3), although the difference was not statistically significant, (Table III). The results of this section are represented in Fig. 9.

When GAD activity in the substantia nigra of the control group was taken as the 100% in order to calculate the percentage decrease in the groups lesioned animals, the percentage decrease was 5-10% greater than when the GAD activity of the substantia nigra of the unlesioned side was taken as a 100%, (Table III). This is due to a non-significant but consistent decrease in GAD activity in the substantia nigra also observed in the unlesioned side of the experimental animals, (Fig. 10, inset).

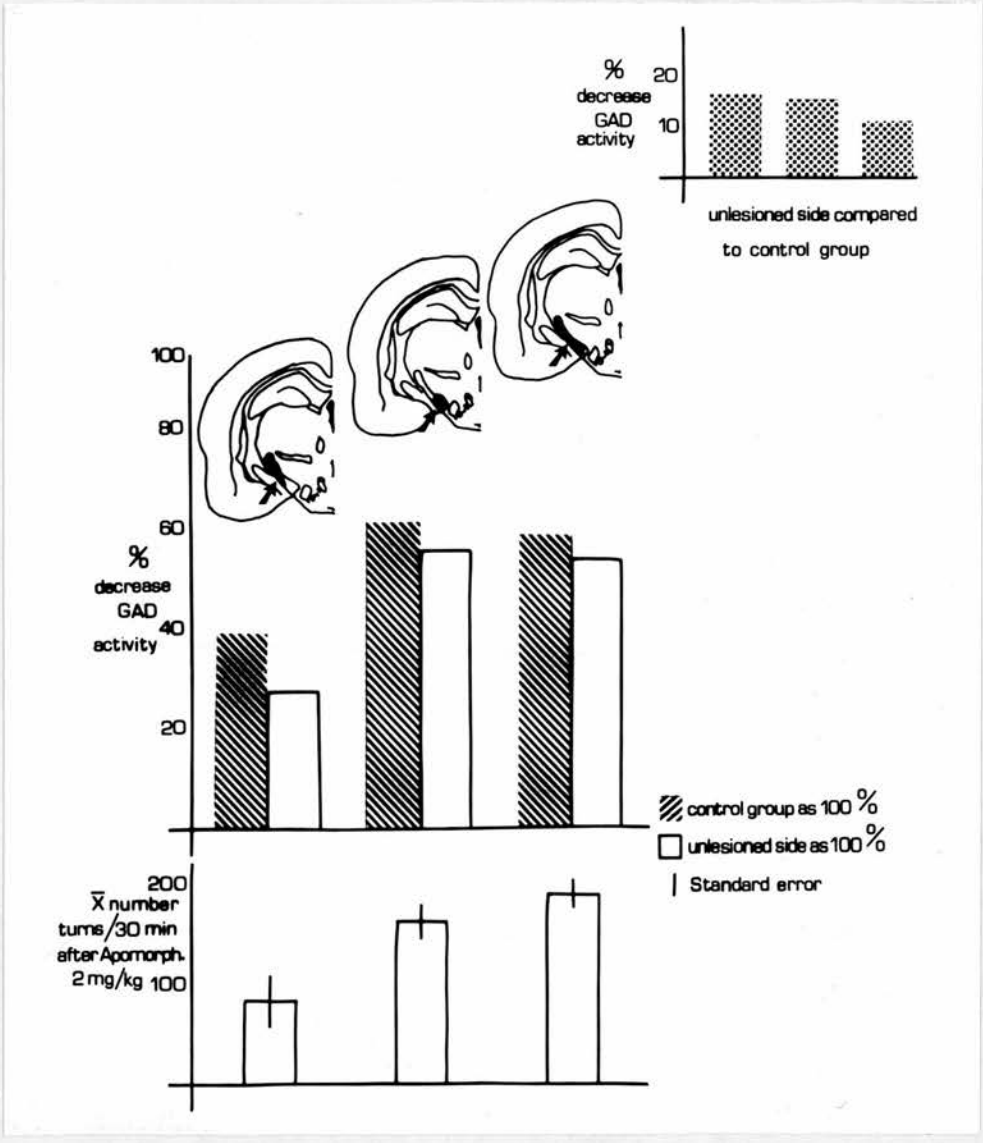


Fig.10 Percentage decrease in GAD activity in the SUBSTANTIA NIGRA for lesions plotted on diagrams reproduced from the Konig and Klippel atlas of the rat brain, (174)

Lower graph; behavioural observation one week after the lesion.

Inset: percentage decrease in GAD activity in the substantia nigra of the unlesioned side of experimental animals compared against the control group.

D3. LESIONS WITH KAINIC ACID AND GAD ACTIVITY DETERMINATIONS.

Kainic acid was injected into 5 rats, aiming for the globus pallidum. After the histological analysis, it was found that only 2 rats had an injection circumscribed to this area, one rat had a lesion of the nucleus ventralis and nucleus reticularis of the thalamus, and 2 rats had the injection in the striatum, one in the ventro-lateral area, and the other in the dorsalateral area of the body of the nucleus, (Fig.11). Comparing the individual values, the lesions of the body of the striatum, showed the biggest decrease in GAD activity in the striatum, substantia nigra and frontal areas. The two lesions in the globus pallidum also produced a reduction in GAD activity in the substantia nigra, (Table IV).

TABLE IV

GAD VALUES AFTER KAINIC ACID INJECTIONS

		SUBSTANTIA NIGRA				STRIATUM			FRONTAL AREAS		
		GAD		%	%	GAD		%	GAD		%
		L	C	C	Con	L	C	C	L	C	C
GLOBUS PALLIDUS	$\bar{X}$	241.2	435.3	44.5	58.2	159.5	170.9	6.7	368.4	327.4	*
	σ	126.4	26.3			37.3	93.3		200.0	196.4	
	N	2				2			2		
STRIATUM	DL	187.1	749.8	75.0	67.6	32.1	212.8	84.9	217.4	384.5	37.6
	VL	598.7	848.1	29.4	*	248.8	487.7	48.9	616.1	586.7	*
THALAMUS		559.1	578.8	3.4	3.0	155.8	193.8	19.6	404.3	406.7	0.6
CONTROL GROUP	$\bar{X}$	576.9									
	σ	112.3									
	N	4									

% Percentage decrease
* Increase
L Lesioned side
C Unlesioned side

Con Control group as a 100%
C Unlesioned side as 100%
DL Dorso-lateral
VL Ventro-lateral

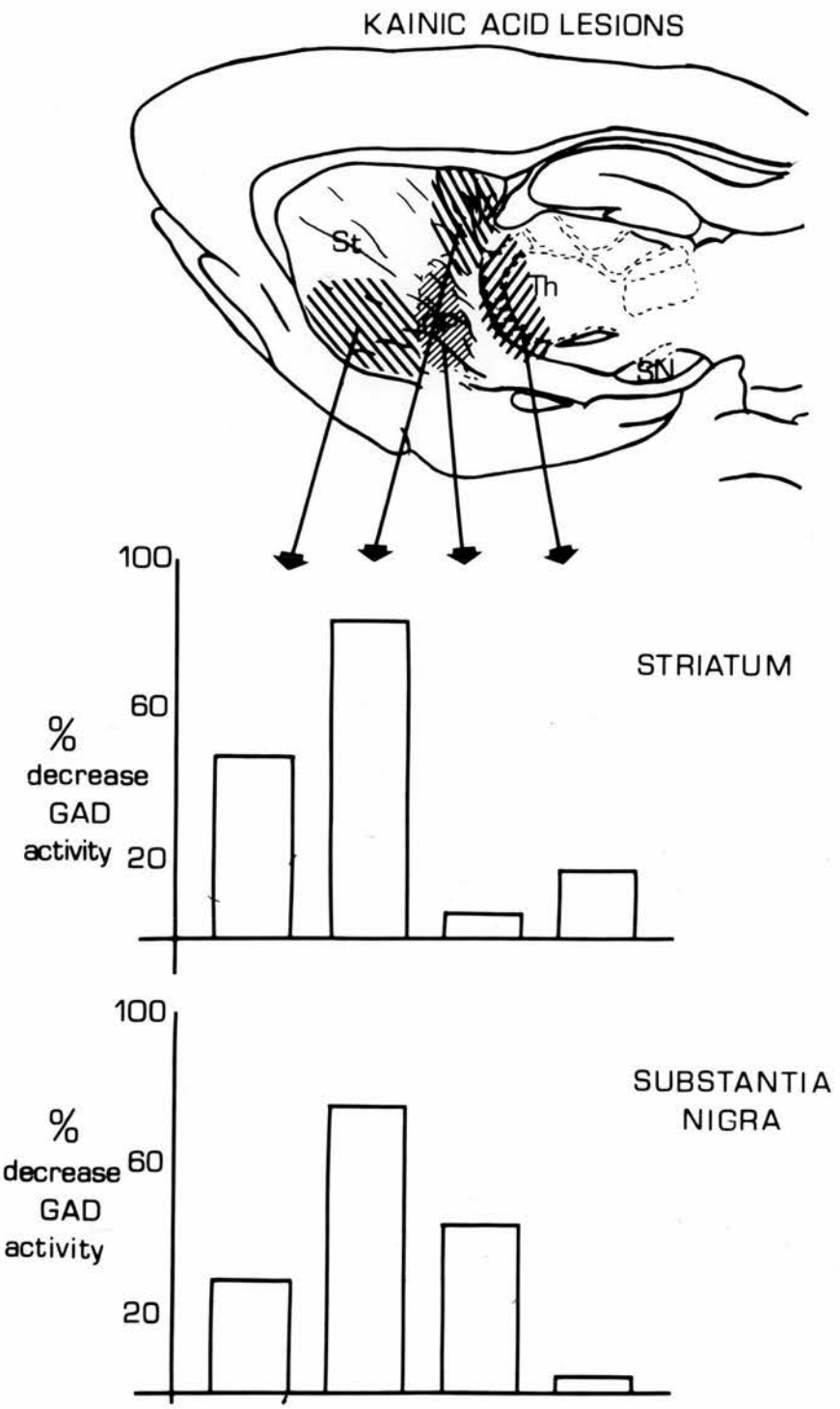


Fig.11 Percentage decrease in GAD activity in the striatum and substantia nigra after kainic acid lesions represented in a diagram of a longitudinal section of the brain taken from the rat atlas, (174).
Unlesioned side as 100%.

TABLE V

STRIATUM

NO DRUG TREATMENT

		DA		HVA		DOPAC	
		L	C	L	C	L	C
Experimental group	$\bar{X}$	8.58	9.14	1.19	1.41	1.04	1.19
	σ	1.98	2.63	0.35	0.38	0.18	0.32
	N	41		8		8	
Control Group	$\bar{X}$	8.97		1.09		1.14	
	σ	2.46		0.26		0.21	
	N	38		12		12	
Unsuccessful Lesions	$\bar{X}$	9.35	8.82	1.52	1.35	0.99	0.96
	σ	2.33	2.74	0.19	0.17	0.13	0.12
	N	12		4		4	

L Lesioned side

C Unlesioned side

D3.1 POST-OPERATIVE OBSERVATIONS.

Lesions damaging the striatum or globus pallidum with kainic acid, produced aphagia which was overcome by giving the rats a special diet of bread and milk for three days after which they started to eat their ordinary food. A week after the operation they showed a decrease in weight between 50-100g compared to their weight on the day of the operation. Afterwards they gained weight normally. The significance of the results is discussed in Chapter IV.

D4./

D4. PARTICIPATION OF THE STRIATO-NIGRAL PATHWAY IN THE METABOLISM OF DOPAMINE.

Rats which had a ventro-medial lesion in the crus cerebri like the one illustrated in Fig.7 were included in this analysis, a small number of animals with lateral lesions (Fig.9) were also included.

D4.1 THE CONCENTRATION OF DOPAMINE AND ITS METABOLITES HVA AND DOPAC IN THE STRIATUM OF LESIONED RATS.

The concentration of Dopamine, HVA and DOPAC were measured in lesioned and control rats after apomorphine and haloperidol. The concentration of dopamine was also determined after alph-methyl-para-tyrosine (AMPT) and its combined treatment with haloperidol (Section C). The results are illustrated in Fig. 12.

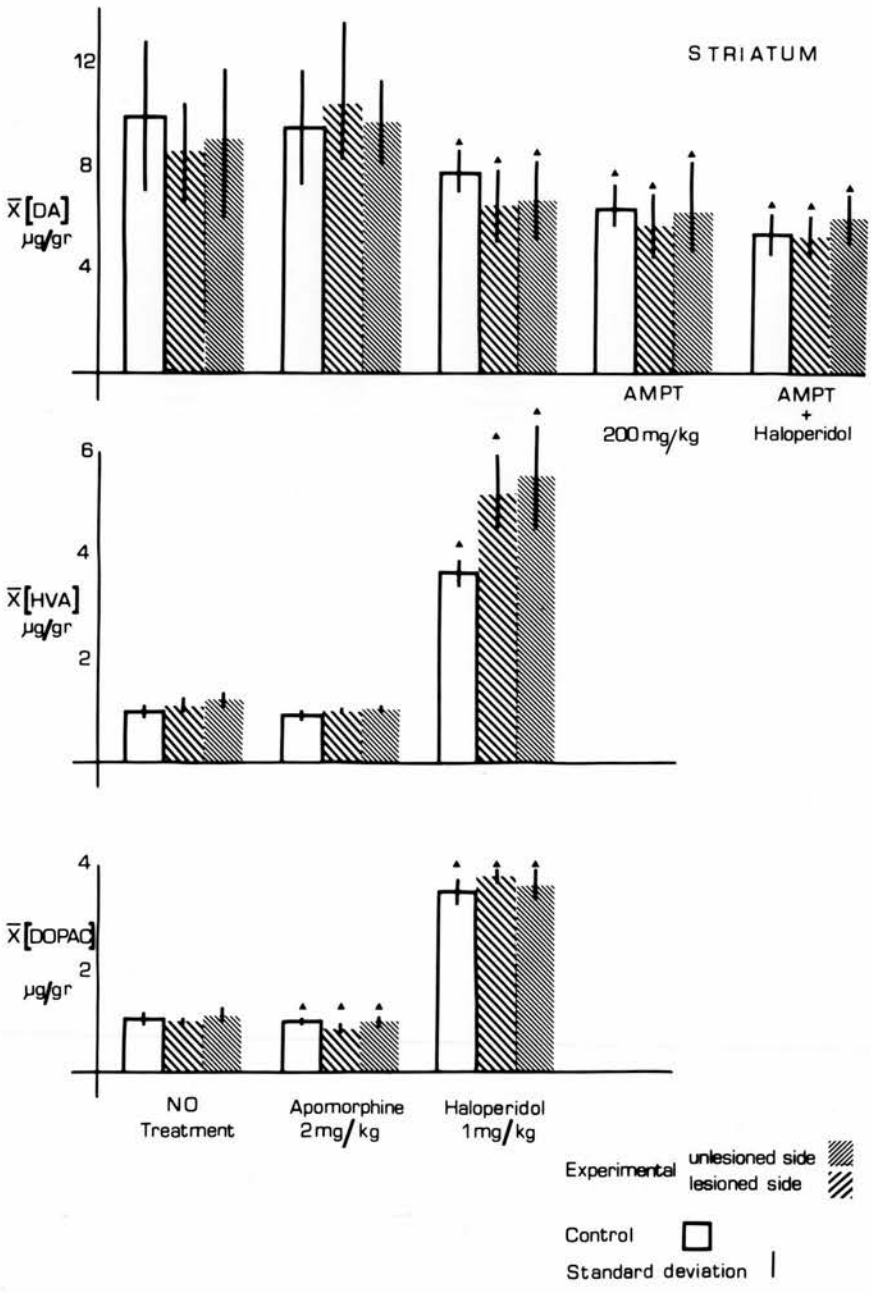


Fig.12 The concentration of dopamine, HVA and DOPAC in the STRIATUM for control and experimental lesioned rats, determined after treatment with different drugs, (Section D4.1). ▲, statistically significant compared with the control group of untreated animals.

TABLE VI

STRIATUM

APOMORPHINE TREATMENT

		DA		HVA		DOPAC	
		L	C	L	C	L	C
Experimental Group	$\bar{X}$	10.38	9.17	1.05	1.11	0.87	0.99
	σ	3.19	1.69	0.08	0.05	0.13	0.19
	N	8		8		8	
Control Group	$\bar{X}$	9.54		0.94		0.95	
	σ	2.25		0.09		0.11	
	N	14		14		14	
Control Group No Treatment	$\bar{X}$	8.97		1.09		1.14	
	σ	2.46		0.26		0.21	
	N	38		12		12	

P<

0.01 ------

0.02 ~~~~~

D4.1.1 NO DRUG TREATMENT.

Lesioned rats had a similar concentration of dopamine in the striatum of the lesioned side compared with the unlesioned side or compared with the concentration of dopamine in the striatum of the control animals, (Table V).

Lesions in other areas around the crus cerebri (unsuccessful lesions) like hippocampus, amygdala, zona incerta or Forel's fields, had a similar concentration of dopamine to the experimental or control groups, (Table V).

TABLE VII

STRIATUM

HALOPERIDOL TREATMENT

		DA		HVA		DOPAC	
		L	C	L	C	L	C
Experimental Group	$\bar{X}$	6.51	6.77	5.24	5.54	3.83	3.14
	σ	1.70	1.55	1.54	2.01	0.21	0.76
	N	19		4		4	
Control Group	$\bar{X}$	7.88		3.69		3.51	
	σ	0.84		1.61		0.56	
	N	20		8		8	
Control Group No Treatment	$\bar{X}$	8.97		1.09		1.14	
	σ	2.46		0.26		0.21	
	N	38		12		12	

P<0.01 --------

D4.1.2 APOMORPHINE TREATMENT.

The concentration of dopamine in the striatum of lesioned rats injected with apomorphine was NOT statistically significant from the concentration determined in both striatal nuclei of control treated rats. Neither group differed statistically from control animals which did not receive any injection. The same results were obtained for the concentration of HVA. The concentration of DOPAC in both experimental and control injected animals was statistically reduced compared with control untreated rats. (Table VI.).

D4.1.3 HALOPERIDOL TREATMENT.

Haloperidol produced a significant decrease in the concentration

of/

of dopamine in the striatum of lesioned and control rats and a significant increase in HVA and DOPAC, compared with control untreated rats, (Table VII). There was no statistically significant difference within any of the groups, only between the haloperidol injected rats and the control untreated animals.

D4.1.4 AMPT TREATMENT.

AMPT produced a significant decrease in the concentration of dopamine in the striatum of treated control or lesioned animals compared with control untreated rats. Similar results were observed for frontal areas. (Table VIII).

There was no statistically significant difference in the concentration of dopamine of the lesioned side compared with the unlesioned side of experimental treated rats or between these values and control AMPT injected animals. These groups were statistically different only when compared against control untreated rats, (Table VIII).

D4.1.5 COMBINED TREATMENT OF AMPT PLUS HALOPERIDOL.

The concentration of dopamine in the striatum and frontal areas of control and experimental lesioned animals treated with these drugs, did not differ compared among themselves, but showed a significant decrease compared with control untreated rats or control rats injected with haloperidol alone, (Table VIII).

D4.2 THE CONCENTRATION OF GABA AND THE ACTIVITY OF ITS SYNTHETIC ENZYME GAD IN THE SUBSTANTIA NIGRA OF LESIONED RATS.

The concentration of GABA and the activity of GAD were measured in lesioned and control rats in conditions where no drugs were administered or after haloperidol treatment. The concentration of GABA was also measured after treatment with apomorphine, and GAD activity was estimated after AMPT and its combined treatment with haloperidol. The results summarized on Tables I, IX, and X are illustrated on Fig. 13. In all cases the concentration of GABA and the activity of GAD were significantly reduced in the substantia nigra of the lesioned side compared with the control group for each treatment or against the unlesioned side of the brain of experimental animals, (Table X).

TABLE VIII

STRIATUM

		AMPT				AMPT + HALOPERIDOL			
		DA striatum		DA frontal		DA striatum		DA frontal	
		L	C	L	C	L	C	L	C
Experimental Group	$\bar{X}$	5.71	6.15	0.38	0.49	5.34	5.94	0.27	0.37
	σ	1.30	1.50	0.10	0.13	0.88	0.92	0.07	0.05
	N	6		6		6		6	
Control Group	$\bar{X}$	6.42		0.38		5.38		0.37	
	σ	0.83		0.13		0.80		0.06	
	N	4		4		4		4	
Control Group Haloperidol treatment	$\bar{X}$					7.88		0.69	
	σ					0.84		0.16	
	N					20		4	
Control Group No-treatment	$\bar{X}$	8.97		0.73		8.97		0.73	
	σ	2.46		0.14		2.46		0.14	
	N	38		6		38		6	

P <

0.05 .....
0.02 ~~~~~
0.01 -------

L Lesioned side
C Unlesioned side

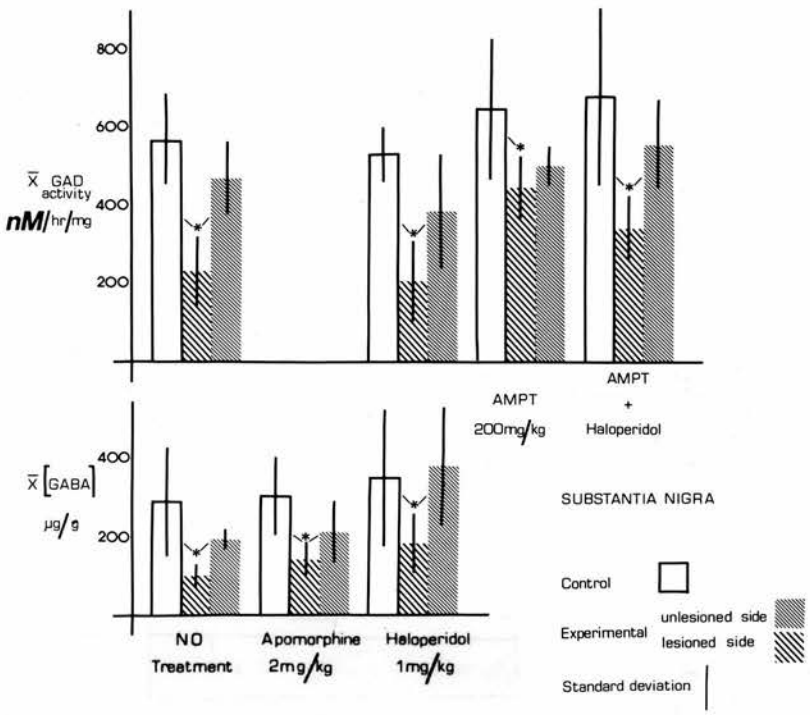


Fig. 13 The concentration of GABA and the activity of GAD in the SUBSTANTIA NIGRA for control and experimental lesioned rats, determined after treatment with different drugs, (Section 4.2) *, statistically significant.

TABLE IX

SUBSTANTIA NIGRA

TREATMENT

		APOMORPHINE		HALOPERIDOL			
		GABA		GAD		GABA	
		L	C	L	C	L	C
Experimental Group	$\bar{X}$	149.0	215.3	203.0	384.8	181.9	387.4
	σ	40.6	87.6	106.0	147.1	77.5	155.8
	N	8		14		10	
Control Group	$\bar{X}$	309.4		537.7		351.10	
	σ	101.5		73.3		178.8	
	N	14		4		6	
Control Group No Treatment	$\bar{X}$	287.3		576.9		287.3	
	σ	141.7		112.3		141.7	
	N	8		4		8	

P<

0.001 – – –

0.01 -----

0.05 •••••

L Lesioned side

C Unlesioned side

TABLE X

GAD ACTIVITY IN SUBSTANTIA NIGRA

TREATMENT		AMPT		AMPT PLUS HALOPERIDOL	
		L	C	L	C
EXPERIMENTAL GROUP	$\bar{X}$	455.3	509.3	340.6	564.3
	σ	81.1	53.0	82.6	113.7
	N	6		6	
CONTROL GROUP	$\bar{X}$	653.7		680.2	
	σ	182.7		250.0	
	N	4		4	
CONTROL Group HALOPERIDOL TREATMENT	$\bar{X}$			537.7	
	σ			73.3	
	N			4	
CONTROL GROUP NO-TREATMENT	$\bar{X}$		576.9		
	σ		112.3		
	N		4		

P<

0.001 - - - -

0.01 -------

0.05 .....

L Lesioned side

C Unlesioned side

E. DISCUSSION.

E1. ABOUT THE METHODS.

Measurements of the contents of GABA in discrete brain regions using different procedures to isolate and cool the brain, have shown that the time elapsed between death and freezing of the tissue is crucial for the determination of the concentration of GABA, (3, 279). In the whole brain a 30-50% increase in GABA content occurs in the first few minutes after death and as much as 100% in brains kept at room temperature for 1 hr, (191). Microwave fixation has been proposed as a method to avoid such increase, (16), however, the time of exposure to microwave seems to be an important variable, and the method itself increases the variance of the results, (3).

Glutamic acid decarboxylase, (GAD), appears to be a better marker for GABA-containing neurons, its activity correlates with the distribution of GABA, (95, 227), it is less subject to post-mortem changes and can be measured in small samples, (278). For this reason GAD activity was mainly used in this study as an index of the concentration of GABA. The concentrations of GABA were also included because the interest of this work was to estimate a decrease in its concentration and not its actual in vivo level or its regional distribution within the brain.

No significant changes in the concentrations of dopamine, HVA or DOPAC, have been found to occur if the time between decapitation and freezing of the tissue is kept between 2-5 min, (207). The dissection procedures as described in Section B3.1, did not take more than 5 min, the same area of both sides of the brain was frozen at the same time and the order in which different areas were frozen was randomized.

E2. GABA AND THE STRIATO-NIGRAL PATHWAY.

The main purpose of this work was to make a lesion in the fibres of the striato-nigral pathway running through the crus cerebri, (282) without damage to the ascending fibres of the nigro-striatal pathway in the medial forebrain bundle, (Chapter I, Section 1.B). The histological analysis indicated that the lesion was circumscribed to the crus cerebri, and this was verified by the biochemical analysis, since no change in the concentration of dopamine or its metabolites, HVA and DOPAC, was observed on the lesioned side, (Table V). Damage to the nigro-striatal pathway has been reported to produce a significant loss in the concentration of dopamine in the striatum, (224) which was not observed after lesions in the crus cerebri.

Anatomical tracing studies indicated that the striato-nigral pathway runs mainly in the ventro-medial portion of the crus cerebri, (282). This was corroborated with these results, since a lesion in the rest of the crus cerebri, including only a portion of the ventro-medial part (lateral area) only produced 39.3% reduction in GAD activity compared to unlesioned animals, (Table III). This reduction was probably due to the partial lesion in the ventro-medial area. This is supported by the fact that a complete lesion of the crus cerebri did not produce a further decrease in GAD activity than the one produced by a lesion in the ventro-medial portion of the crus cerebri.

There are afferent fibres from the globus pallidum to substantia nigra and the pathway which they follow runs very close to the site of the crus cerebri lesion, (Chapter I, Section 1.A)., therefore this lesion will damage both pathways to the substantia nigra, the striato-nigral and the pallido-nigral.

Although/

Although the numbers are very small to conclude anything from the experiments injecting kainic acid, it is relevant to note that both striatum and globus pallidum lesions produce a decrease in GAD activity in the ipsilateral substantia nigra. It would be interesting to verify this in a bigger sample and also determine the concentration of SP in the substantia nigra after lesions with kainic acid in these structures.

It is interesting that the rat with a lesion in the dorso-lateral area of the body of the striatum had the biggest percentage decrease in GAD activity. This is an area which projects mainly to the intermediate portion of the pars reticulata of the substantia nigra, in the rat, (43).

It is relevant to point out that after kainic acid lesions in the striatum there seems to be an increase in GAD activity in the contralateral substantia nigra, if the values are compared against the control group, (Table IV). Since the GAD activity values for the control group and the lesioned animals were not estimated at the same time, there is the possibility of inaccuracy of the biochemical assay, however, this does not seem to be the case, since the GAD activity for the substantia nigra of the intact side of thalamic and globus pallidum injected animals falls within the range of the control values. The increase in GAD activity in the contralateral substantia nigra suggests that there is a relation between the striatum and the substantia nigra on both sides. A connection between the two striatum had been reported in rats, with horseradish peroxidase injections, (205). This connection is one of the many pathways which can be proposed to relate the structures on both sides. Lesions of the crus cerebri produced a non-significant but persistent decrease in GAD activity on the substantia nigra of the intact side, (Table III, Fig.10 inset). This again

agrees/

agrees with the suggestion that somehow both nigral nuclei must be interconnected. It has also been found that dopamine formed from radioactive tyrosine collected from both substantia nigra nuclei change in opposite directions, (215, 219, 216). An influence of one substantia nigra to the other can also be mediated through the nigro thalamic, thalamo cortical projections and from the cortex to both striatum which send afferents back to the substantia nigra, (54).

E.3 DOES THE STRIATO-NIGRAL PATHWAY PARTICIPATE IN THE METABOLISM OF DOPAMINE?

It has been reported that after antipsychotic drugs (e.g. chloropromazine and haloperidol) the concentration of dopamine is significantly reduced and the levels of its metabolites (e.g. HVA) are increased(6, 155). Opposite effects are reported after treatment with dopamine receptor agonists (e.g. apomorphine) where although the concentration of dopamine remains unchanged the levels of HVA are reduced, (253). This has been taken as a strong evidence in favour of the feedback hypothesis. The fact that the blockade or stimulation of dopamine receptors have opposite effects led to the conclusion that the feedback originates from the postsynaptic dopamine receptor activity (Chapter I, Section 2.A).

Haloperidol treated rats to which AMPT was also given showed a non-significant tendency towards an acceleration of dopamine loss. This was explained by a negative feedback mechanism due to postsynaptic receptor blockade, (6).

The results reported here (Section D.4.1; Fig. 12) mainly confirm these observations. The only discrepancy found was that the

—concentration/

concentration of HVA was not significantly reduced after apomorphine, although the concentration of DOPAC was. This may be due to difference in dosage: 25mg/kg subcutaneous, 45 min before death (253), against 2mg/kg, intraperitoneal, 30 min before death used in this experiment. A similar decrease in DOPAC has been reported, (145).

The relevance of this experiment lies in that even animals with lesions of the striato-nigral pathway whose activity was supposed to be sponsible of the observed effects, showed changes in the same direction as control unlesioned rats.

It can be argued that the lesion was not complete and that activity of the fibres left intact is responsible for the results. However comparisons of the lesion site with the anatomy of the striato-nigral pathway (282) suggests that the lesion clearly damages the area where these fibres are contained.

The activity of GAD or the concentration of GABA was estimated in every drug-treated group (Section 4.2, Fig.13) and the histological analysis was done for every animal in order to determine the extent of the lesion. In every case the decrease in GAD activity or in the concentration of GABA was significantly reduced compared to unlesioned rats, and the lesion was found in the ventro-medial area of the crus cerebri.

Alternative explanations for the control of dopamine metabolism have already been discussed (Chapter I, Sections B.2.3.; C.1 and D.1).

The results of this experiment cannot give direct support to any of them but agree with the generaly idea that other explanations for the effects of antipsychotic drugs and dopamine receptor agonists, on dopamine metabolism, must be sought.

A/

A report in agreement with this suggestion has been put forward recently. It has been shown in the rat, that after a lesion of the striato-nigral cells with kainic acid, the systemic administration of haloperidol increases and of apomorphine decreases DOPAC levels equally in both control and lesioned animals in the striatum, (62). This indicated that an action at the level of the post-synaptic dopamine receptors is not a pre-requisite for the effects of these drugs.

It has been reported that radiofrequency lesions in the striatum and globus pallidum produce not only a decrease in GAD activity but also in the concentration of SP, (160). Lesions interrupting the fibres from these nuclei to the substantia nigra, confirm these results, (Table II), suggesting that this fibre system includes SP-containing fibres as well as GABA containing ones.

Recently, release of SP has been observed in vitro from the substantia nigra, (153). An interaction between GABA and SP terminals is proposed, since addition of GABA to the superfusion media inhibited the release of SP, (153). However, the role of SP in the substantia nigra remains to be studied.

Since lesions of the striato-nigral pathway reduced the concentration of GABA as well as SP, it is not likely that SP is involved in the control of dopamine metabolism. SP and GABA could be involved respectively in the excitatory and inhibitory responses recorded from cells in the substantia nigra after stimulation in the striatum. SP and GABA could be mediating different kinds of output from the striatum to thalamus via the substantia nigra, since substantia nigral cells in pars reticulata are known to project to thalamic nuclei, (Chapter IV, Section E.).

The/

The feedback hypothesis predicted that antipsychotic drugs blocked dopamine receptors on GABA containing neurons in the striatum producing a decrease in the concentration of GABA in the substantia nigra relieving dopamine cells from inhibition. Dopamine cells in turn according to the hypothesis would increase their activity and the synthesis of dopamine giving as a result that significant increase in dopamine metabolites in the striatum observed after antipsychotic drugs. However, against prediction the decrease in the concentration of GABA and in GAD activity observed after lesions in the striato-nigral pathway, does not intervene in any way in the response produced by dopamine cells to haloperidol or apomorphine.

A strong evidence in favour of the participation of GABA according to the feedback hypothesis, was the reduction of haloperidol induced activation of TOH in the striatum after injection of GABA agonist, muscimol, in to the substantia nigra, (135). However, since this experiment involved an injection directly into the substantia nigra through an implanted cannula, damage to nigral cells could account for the results.

Apart from the so-called striato-nigral pathway, there are at least two other pathways from the striatum to the substantia nigra which could be involved in a feedback loop. One involves the tail of the striatum, (282, 43). This pathway has been shown to travel lateral to the pathway from the body of the striatum, (282), lesions of the crus cerebri in this area, (lateral lesions, Fig.9), interrupt these fibres without effect on dopamine metabolism measured by the concentration of HVA and DOPAC in the striatum with no drug treatment or after haloperidol or apomorphine administration, (D.4.1). Therefore, the participation of this pathway is/

is eliminated. Another possible pathway involves connections from the striatum to the subthalamic nucleus and from there to substantia nigra, (140). The participation of this pathway can be also excluded, since lesions of the crus cerebri in several cases included the subthalamic nucleus without any effect on dopamine metabolism.

F. SUMMARY.

I. It was observed that lesions of the striato-nigral pathway in the ventro-medial portion of the crus cerebri produced:

A - In the striatum of the lesioned side: No change in the concentration of dopamine, HVA and DOPAC. Indicating that the nigro-striatal fibres were not damaged.

B - In the substantia nigra of the lesioned side: A significant decrease in the concentration of GABA, in the activity of GAD and in the concentration of SP.

C - In the substantia nigra of the unlesioned side: A non-significant but consistent decrease in GAD activity.

II. A lesion of the striatum and of the globus pallidum with kainic acid also produced a significant decrease in GAD activity in the ipsilateral substantia nigra.

III. Drug Treatment: In the striatum of control and lesioned animals:

A - Apomorphine produced a significant decrease in the concentration of DOPAC.

B - Haloperidol produced a significant increase in the concentration of HVA and DOPAC and a significant decrease in the concentration of dopamine.

It/

It is suggested that the striato-nigral pathway is not involved in the control of dopamine metabolism.

CHAPTER III

ELECTROPHYSIOLOGY OF THE SUBSTANTIA NIGRA PARS COMPACT AFTER A LESION IN THE STRIATO-NIGRAL PATHWAY.

CHAPTER III

ELECTROPHYSIOLOGOY OF THE SUBSTANTIA NIGRA PARS COMPACTA AFTER A LESION IN THE STRIATO-NIGRAL PATHWAY

A. INTRODUCTION.

Strong support for the feedback hypothesis came from electrophysiological experiments, (Chapter I, Section E.3.2). However, not all the results agree with the hypothesis. Iontophoretic administration of apomorphine onto the substantia nigra cells in the pars compacta decreases their firing rate, (1) and direct microinfusion of amphetamine also has a depressant effect on these cells, (129). This suggested that these drugs when injected intravenously were having both a direct and indirect effect. That is they were acting in the striatum but also in the autoreceptors of substantia nigra cells, (Chapter I, Section E.3.2). It seemed that one way of finding out if activity of striatal cells sending efferents to the substantia nigra were involved in the response to drugs, was to make a lesion of the striato-nigral fibres and test the response of dopamine cells to drugs. However, in the experiments reported in the literature so far, the extent of the lesions seemed to affect in one way or another the dopamine cells, (40, 42, 130). Since the results presented in Chapter II showed that our lesions do not affect the biochemical response of dopamine cells of the substantia nigra it was decided to make a localized lesion in the striato-nigral pathway in order to test the effect of the intravenous administration of amphetamine and haloperidol on cells in the substantia nigra pars compacta.

The results reported here suggest that lesions of cells in the striatum with kainic acid or electrolytic lesions of the striato-nigral pathway/

pathway do not prevent the effect of amphetamine or haloperidol except when the lesion extends to the substantia nigra. In order to give further evidence, partial lesions of the substantia nigra pars compacta were made with 6-hydroxydopamine. In this case the effects of the amphetamine on the remaining intact cells were also prevented. This suggests that all dopamine cells must be intact in order to observe the decrease in the firing rate produced by the administration of amphetamine, whereas efferent fibres from the striatum do not mediate this response.

B. PROCEDURE.

Rats were lesioned in the striatum or in the striato-nigral pathway, allowed to recover for a week and tested for turning behaviour. The recording experiment was done 10-15 days after the lesion. In some occasions a stimulating electrode was implanted before the recording to attempt to identify the cells antidromically.

Other rats were lesioned acutely during the recording experiment in which case they did not have any previous treatment.

Only one cell was recorded per experiment to avoid the residual effect of drugs. The firing rate of the cell was tape-recorded and plotted on paper. After a control period, amphetamine was injected intravenously, later, in some cases, haloperidol was administered followed by a second injection of amphetamine.

At the end of the recording, pontamine blue dye was ejected from the tip of the electrode to identiy the recording area. Then the animals were sacrificed and the brain removed for histological analysis.

C. METHODS.

The procedure for the stereotaxic surgery and the electrolytic lesions/

lesions has been described in Chapter II, Sections, C.1, C.1.1.

C.1 KAINIC ACID INJECTIONS

This procedure has been described in Chapter II, Section C.1.2, except that the co-ordinates in this case were aiming for the striatum.

According to the Konig and Klippel atlas, (174):

Antero-Posterior:	+ 7.4
Lateral:	- 2.5
Vertical:	- 4.5

With the surface of the dura membrane as the reference point for the vertical co-ordinate.

C.2 6-HYDROXYDOPAMINE (6-OHDA) INJECTIONS

6-OHDA, (Hassle, Biotec) 1.25mg/ml was dissolved in saline, 0.9% (w/v) containing 1.0mg/ml of ascorbic acid, and the pH was adjusted to 7.4.

A stainless-steel needle, for cartridge syringes, gauge 30, 0.46 mm in diamter was lowered into the brain aiming for the fibres in the medial forebrain bundle, at the following co-ordinates, (174):

Antero-Posterior:	+ 2.9
Lateral:	- 1.2
Vertical:	- 8.0

The volume injected was 4 µl over a period of 5 min. To allow for diffusion, the needle was left in place for further 5 min.

C.2.1 BIOCHEMICAL ANALYSIS.

In order to determine the extent of the 6-OHDA lesion, the concentration of dopamine in the striatum was determined with the assay described in Chapter II, Section C.3.2.

C.3 POST-OPERATIVE OBSERVATIONS:

These were the same as described in Chapter II, Section C.2, except that the dose of apomorphine administered intraperitoneally to the 6-OHDA lesioned animals was 0.2 mg/Kg. To select rats which only had a partial lesion of dopamine cells, only those which turned after being handled and not more than 30 times in 30 min were selected for the recording experiment.

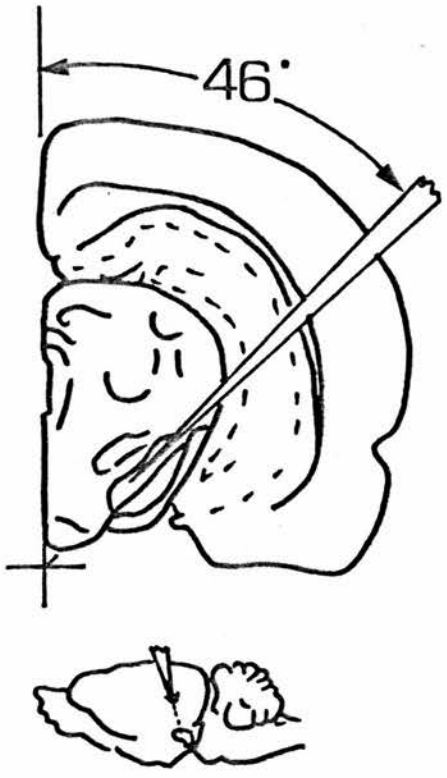


Fig. 14 The lateral approach used in the recording experiments.

C.1 ACUTE EXPERIMENTS.

C.4.1 SURGICAL PROCEDURE.

The rats weighted between 210-250g at the time of the experiment. They were anaesthetized as described in Chapter II, Section C.1. Once the flexor reflexes were lost, a tracheostomy was performed and a glass tracheal cannula fixed, through which the anaesthetic fluothane/air mixture was delivered from then on at a rate of 1.5% 300ml/min until the beginning of the recordings, when the rate was lowered to 0.7%. If the tracheal cannula blocked during the experiment, it was cleared by suction with a plastic cannula and a syringe. Once the neck wound was closed the rat was placed on a homeothermic blanket, (240 Epil), maintained at 37'C, and fixed to a David Kopf stereotaxic frame. When a blood pressure recording was done, the femoral artery was cannulated, the cannula (Portex, O/D 0.5mm) was flushed through with 5.000 u/ml Heparin (Evens) solution and filled with 1:1000 u Heparin solution.

As suggested in the literature, (100, 103, 43) the recording electrode was introduced into the brain at an angle of 46' from the vertical line to ensure that the electrode lies along the longitudinal axis of the substantia nigra, increasing the possible number of cells available for recording, (Fig. 14).

On the recording side the temporalis muscle was pushed away from the skull. The stereotaxic co-ordinates, (174) aiming for the substantia nigra pars compacta were:

Antero-Posterior:	+2.3
Lateral:	-6.8 to -7.2 (according to the weight of the rat)
Vertical:	-6.0 to -8.0

The co-ordinates were measured using a fine pin placed instead of

the microelectrode. A hole approximately 3mm in diamter was bored for the recording electrode. In order to leave the cortical surface intact, a microscope at low magnification was used during this procedure, and a thin piece of bone was left to be removed with fine forceps. Then the dural membrane was ruptured with a small needle and lifted to the side with forceps. The exposed cortex was bathed in warm liquid paraffin to prevent drying of tissue. A drawing of the exposed cortical surface was made indicating the area where the electrode had to be placed.

C.4.2 IMPLANTATION OF ELECTRODES.

In some occasions an electrode for stimulation or lesioning, was implanted. It was made of twisted stainless steel wire, 0.16 mm in diameter and insulated except for the tip.

The stimulating electrode aiming for the fibres of the medial forebrain bundle was placed using the co-ordinates described in Chapter II Section C.2. The electrode used for lesioning was placed in the ventro-medial portion of the crus cerebri using the co-ordinates described in Section C.1.1.

The electrode was fixed to the skull with dental acrylic (Howmedica Internal. Ltd.). To provide support a nylon screw (Walker-Spencer Component Ltd.) was inserted into a hole drilled in the skull at the level of the lambda suture.

C.4.3 MICROELECTRODES.

A theta style cappillary glass micropipette, (TGC 150, Clark Electromedical Instruments) was used for making the microelectrodes. The glass was pulled with an electrode puller (Forth Instruments Ltd) and/

and the microelectrodes were filled using a syringe and a long fine needle with pontamine sky blue (Gurr, Ltd.) made up as a 2% solution in 0.5 M sodium acetate. The solution was filtered through a medium fast paper filter, (Qualitative No. 1, Whatman), and the pH adjusted to 7.7.

The resistance was determined using the method which relies on generating a constant current signal that produces a voltage across the microelectrode, proportional to the electrode resistance, (37). Microelectrodes having a resistance of 10-20 megaohms were used.

The electrode held in a special adapted David Kopf electrode holder was placed in position with the help of the microscope. A small stepping motor, (Forth Instruments Ltd.) fitted into the holder connected to a microdrive was used to advance the electrode by 2μm steps.

C.4.4 RECORDING SET UP.

4.4.1 RECORDING OF CELLS.

The electrode was connected to the gate of a field-effect transistor (FET No. 2N3819, R.S. Components Ltd.) used as a voltage follower, by means of a silver/chloride wire. The voltage for the FET came from two 6 V batteries connected in series. The output of the source was connected to a differential amplifier (5A22N; Tektronix) of a dual-beam storage oscilloscope (D.13, Tektronix). The reference point was provided from the indifferent electrode made of a silver/ silver chloride wire inserted into a neck muscle. The bandwidth of the amplifier was set at 1.0-10.0 KHz.

An earth lead was connected to the rat's skin by means of a small crocodile clip. The earth of all the recording equipment was referred to a copper water pipe. With these recording conditions, the signal to/

to noise ratio fluctuated between 1:5 and 1:10.

The output from the amplifier was also connected to a Spike Processor (D.130, Digitimer), and to a tape recorder (Nivico, Victor Co.). The output of the tape recorder was monitored on an oscilloscope (Devices 312-, Digitimer). The windows of the spike processor were also displayed in this oscilloscope which also received the signal from the amplifier. The spike processor was set to count the number of spikes crossing the window every 10 sec and its output was connected to a Potentiometric recorder,(R.e.20, Servoscribe, Goerz, Electro.). The action potentials were recorded using a window discriminator whose output pulses were led to the input of a GEC 2050 computer terminal.

4.4.2 BLOOD PRESSURE RECORDING.

The cannula inserted in the femoral vein was connected to a transducer (4-327-L221, Bell & Howell Ltd.) and the blood pressure recorded on a UV recorder (Bell & Howell Ltd.).

4.4.3 STIMULATION.

When a stimulus was delivered, an isolated stimulator (2533, Devices) was used connected to a current source (NL800 NeuroLog System) set up with a maximum scale of 1 mA. The stimulator was triggered with a Digitimer (3290, Devices).

4.4.4 LESIONING

A current provided by a 6 V battery measured with an ammeter connected in series, passed through the electrode for a period of time such that an electrolytic lesion was made using 6 mCoulombs.

C.4.5 DRUGS ADMINISTERED.

DRUG	DOSE	CONCENTRATION
D-Amphetamine (Smith Kline & French Co. Ltd.)	1.0mg/kg	1.0mg/ml
Haloperidol (Serenace, Searle & CO.)	0.1mg/kg	0.1mg/ml

A tail vein was used for the injection, the skin covering the blood vessel was pulled away and the injection was done under microscopic guidance.

C.5 DATA ANALYSIS.

The computer was set to build an interval histogram which was shown in its display monitor and plotted on paper. The interval histogram had a time base of 10 or 100 msec for its 256 byte samples, and counted 1.000 spikes.

When a stimulus was delivered, a post stimulus histogram and an averaged evoked potential were built with the computer.

The statistical significance of the effect of a drug was estimated with a Chi Square test (26). The frequencies were calculated by playing back the recorded experiment into the spike processor, set to count continuously. The number of spikes during 5 min before the injection of a drug were considered the expected frequency, and the number of spikes during the first 5 min after the injection were considered the observed frequency.

C.6 IDENTIFICATION OF CELLS.

The following 3 criteria aid the choice of cell during the experiment, but all cells were positively identified after the experiment by histological analysis.

1/

1 - The duration of the spike was used to discard action potentials recorded from axons. The minimum duration of a spike to be considered as recorded from a cell body, was 2 msec.

2 - The firing rate of dopamine cells ranges between 3-10 spikes/sec, (Chapter I Section 3.D). Only spikes falling in this range were analysed.

3 - The interval histogram with a mode of 60-250 msec has been considered also a criteria to identify these cells (Chapter I, Section 3.D). Therefore, an interval histogram was always analysed before continuing with the experiment.

Attempts to identify the dopamine cells by recording antidromic spikes after the stimulation of the fibres of the medial forebrain bundle were also made.

Pontamine blue dye was always ejected from the tip of the electrode at the end of the experiment, in order to identify the recording area. To eject the dye, 10 μA/5 min (negative current) were passed between the electrode and the earth lead, (145).

C.7 HISTOLOGICAL ANALYSIS.

The extension of the lesion was observed using the method described in Chapter II, Section C.4.

The mark left by the ejected dye was seen during sectioning on the microtome with the help of a magnifying glass. The sections were collected on glass microscope slides and left in the vapour of formaldehyde/

formaldehyde solution (AnalaR, BDH Chemicals Ltd.) for 24 hr. Then the staining of the tissue was carried out as previously described (Chapter II, Section B.4.1).

C.7.1 FLUORESCENCE MICROSCOPY.

The fluorescence of dopamine cells and of the pontamine blue dye, was observed in some occasions, to determine the recording site.

At the end of the experiment the rats were perfused through the ascending aorta with 150 ml of an ice-cold solution containing 2% (w/v) glyoxylic acid monohydrate and 0.5% (w/v) paraformaldehyde plus 40g magnesium sulphate at pH 4-5, (190). The rat brain was dissected out and prepared for fluorescence microscopy according to the method described by Marsden and Kerkut(196). A piece of tissue 2.5 mm thick, containing the substantia nigra was frozen in iso-pentane (BDH, Chemicals Ltd.), cooled to the temperature of liquid nitrogen, and stored in liquid nitrogen not more than 2 weeks. The tissue was then freeze-dried, treated with paraformaldehyde vapour, embedded in paraffin in vacuo and sectioned. The paraformaldehyde vapour treatment was performed at 80' C for 1 hr using paraformaldehyde (5g/l) equilibrated at 69.2% relative humidity. The sections were mounted in Entellan (Merck) and examined and photographed using a fluorescence microscope, (Carl Zeiss).

In a few occasions the animals were not previously perfused in which cases the brain was dissected and prepared for fluorescence immediately after killing the animals.

D. RESULTS.

D.1 HISTOLOGY.

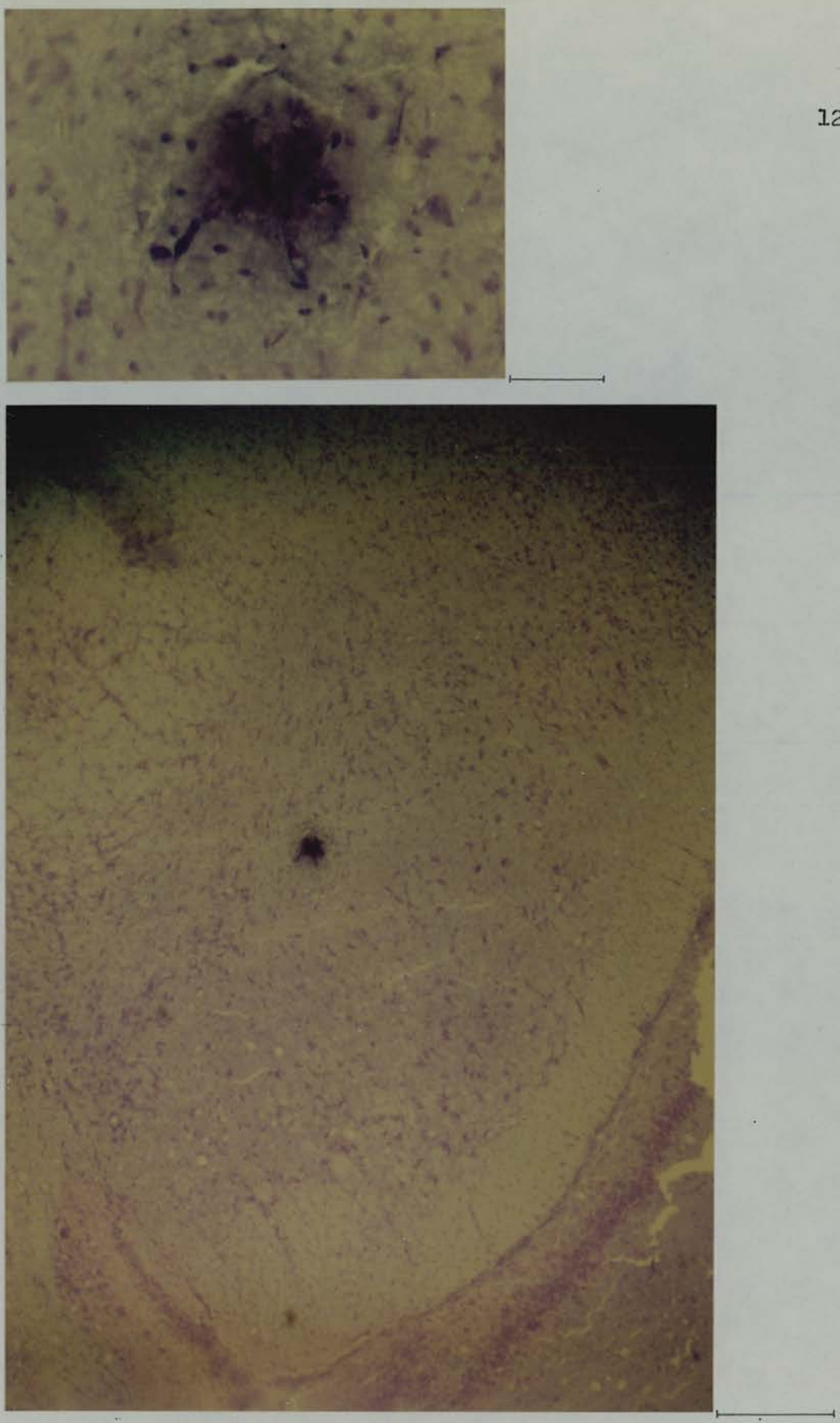
The localization of the recording area was done for every animal, and/

and only those showing a dye spot in the substantia nigra pars compacta, as illustrated in Fig. 15-16 were considered as successfully recorded from this area.

The fluorescence microscopy did not give successful results every time and since it is a time consuming procedure, only 4 rats were finally analysed with this technique, an example of the dye spot around the fluorescent dopamine cells is given in Fig. 17. It was confirmed (190) that perfusion with glyoxylic acid offers an improved visualization of dopamine neurons, (Fig. 18) compared with the fluorescence observed when the animal was not perfused, (Fig. 17).

D.2 CONTROL GROUP.

This group was composed of 4 rats with no previous treatment. After amphetamine the firing rate of dopamine cells decreased significantly in all of them, (Fig. 19). To one animal, haloperidol was given approximately 30 min after the amphetamine injection and the firing rate was significantly increased compared against the firing rate before and after the injection of amphetamine. When amphetamine was administered after haloperidol, it did not alter the discharge of the cell, (Fig. 19).



ig. 15 Dye ejected from the recording electrode localized in the SUBSTANTIA NIGRA PARS COMPACTA. Staining: Kluver and Barrera Technique, (Section C.7). Calibration mark: Top = 50µm, Lower: 100µm.

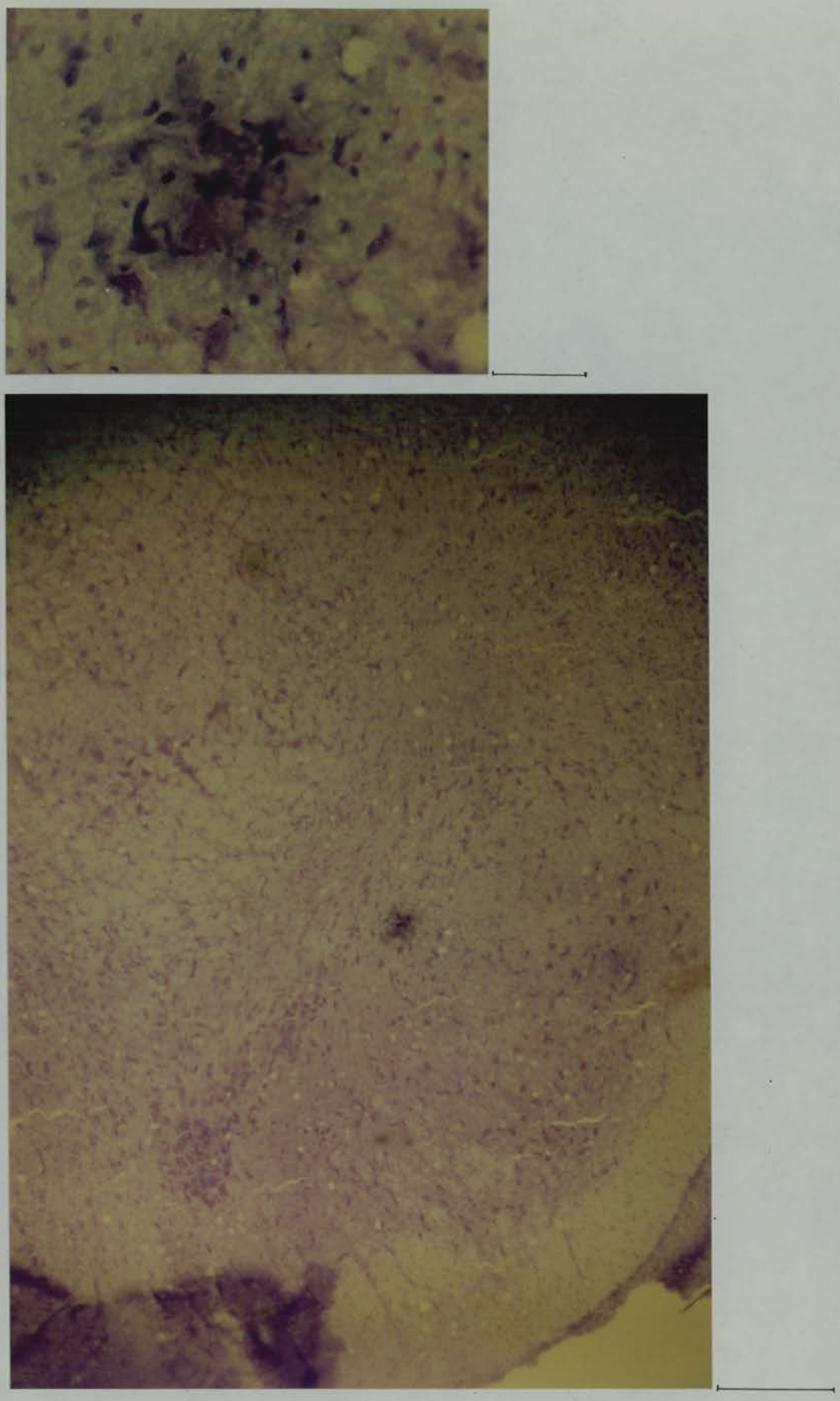
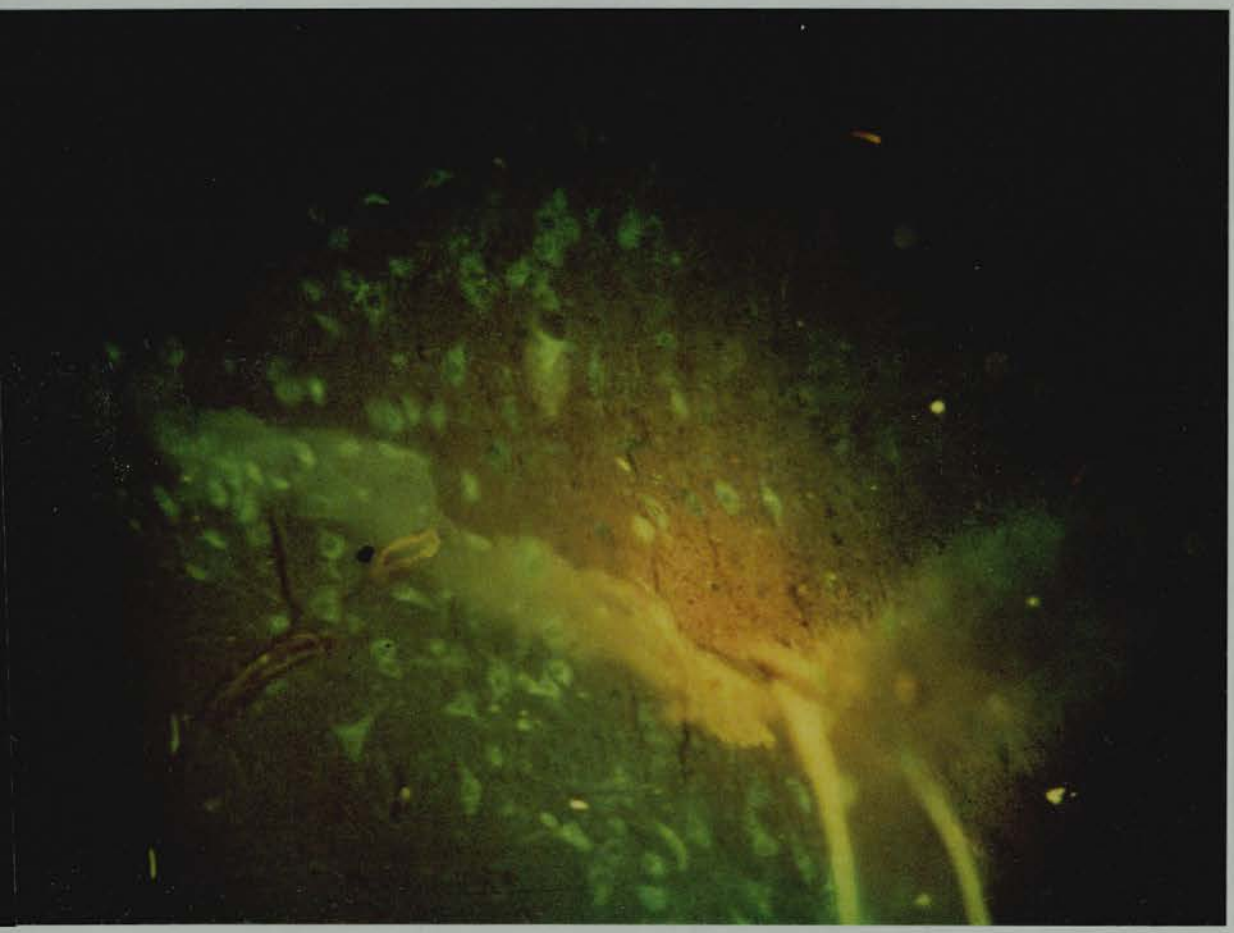
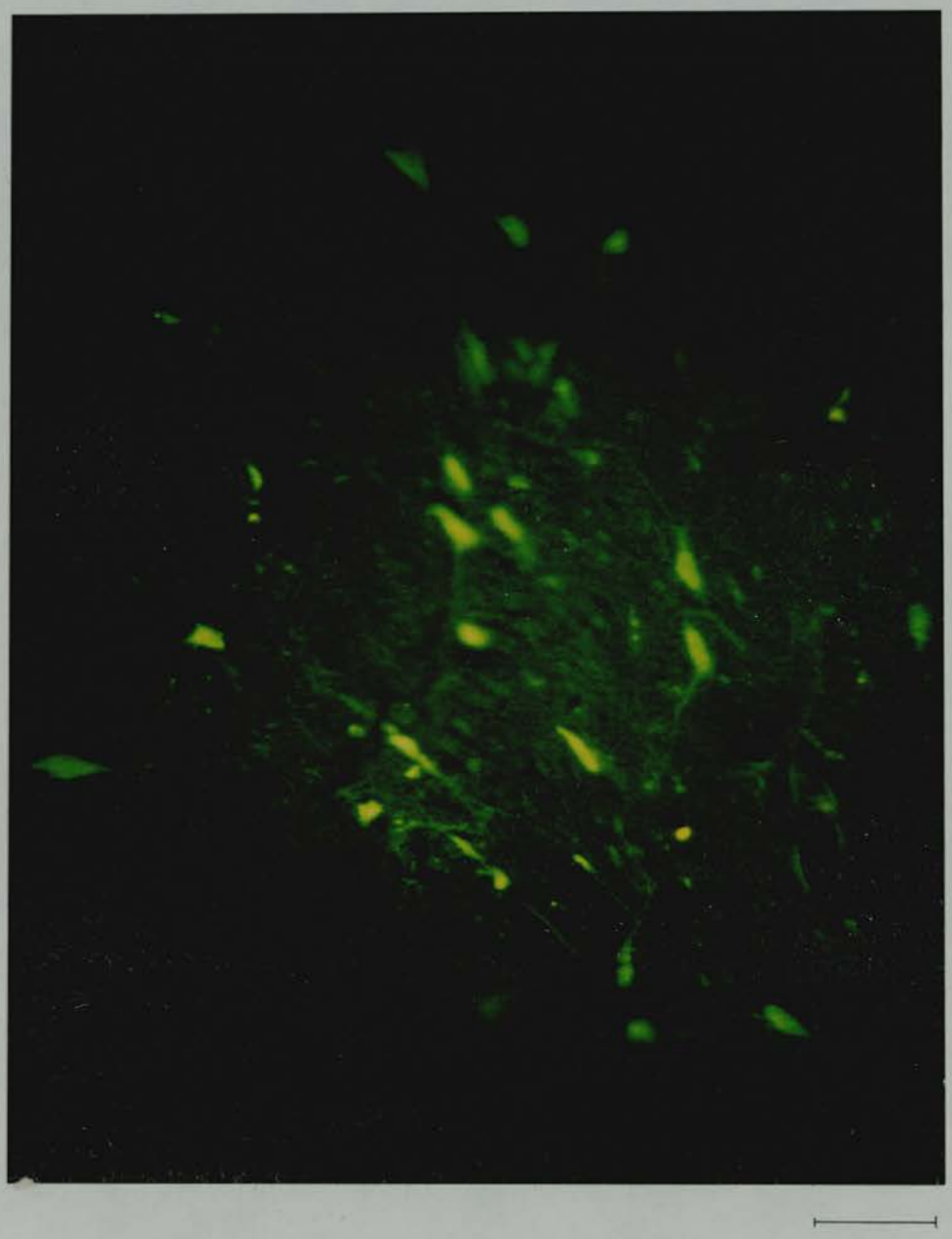


Fig. 16 Dye ejected from the recording electrode localized in the SUBSTANTIA NIGRA PARS COMPACTA. Staining: Kluver and Barrera Technique (Section C.7). Calibration Mark: Top = 50µm, Lower: 100µm.



ig. 17 Dye spot in the SUBSTANTIA NIGRA PARS COMPACTA, after treatment for fluorescence microscopy, (Section C.7.1).
Calibration Mark: 50µm.



ig. 18 Fluorescence of dopamine cells after perfusion with Glyoxylic Acid, (Section C.7.1).

Calibration mark: 50 µm.

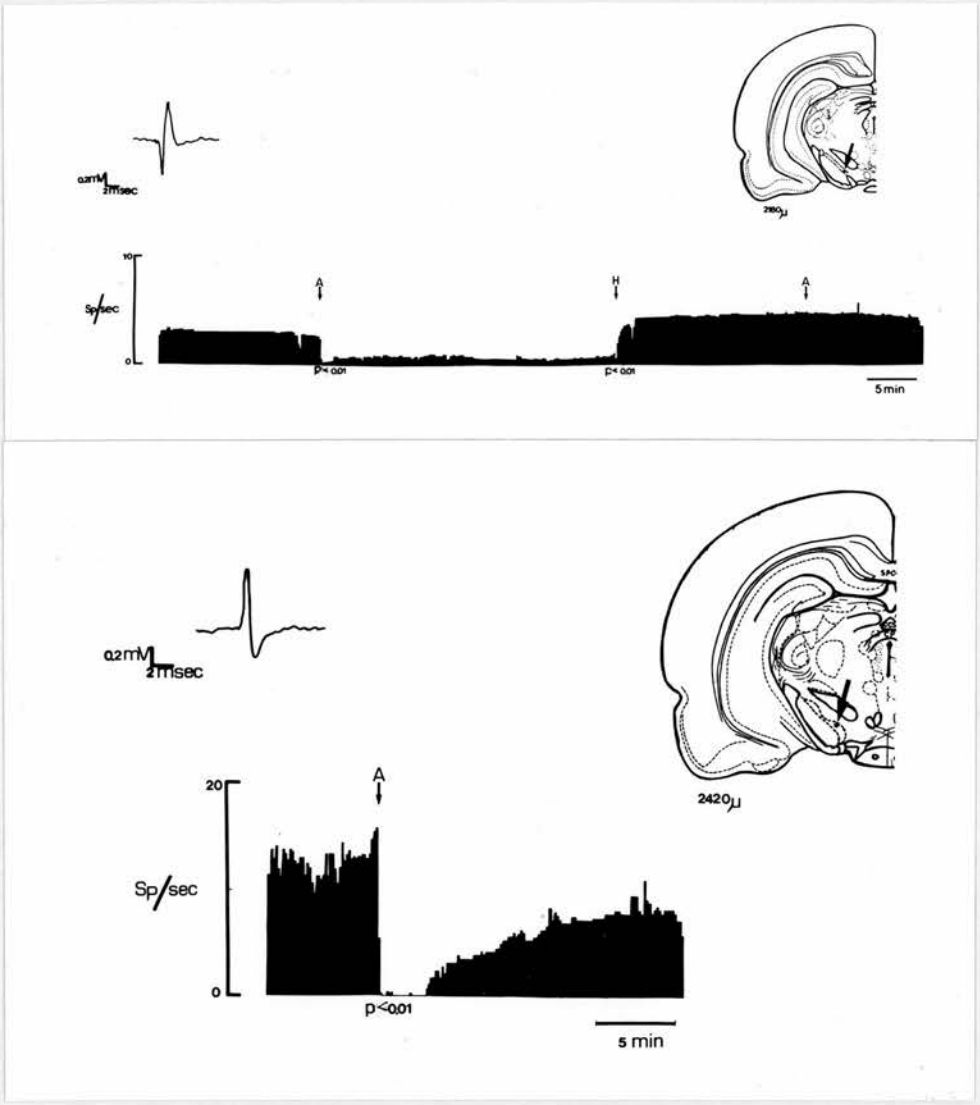


Fig. 19 Recording of the firing rate of a cell in the SUBSTANTIA NIGRA PARS COMPACTA IN CONTROL ANIMALS, (Section D.2).

Right Corner: Recording site, (arrow)plotted on a diagram reproduced from the Konig and Klippel Atlas of the rat brain, (174).

Left Corner: Spike reproduced from a photograph taken during the recording.

Lower Trace: Firing rate of the cell and the effect of: A = Amphetamine (1mg/Kg) and H = Haloperidol, (0.1 mg/Kg), administered intravenously.

Spikes recorded from an area outside the substantia nigra did not alter the firing rate after the administration of amphetamine. An example is shown in Fig. 20.

In two control rats, the blood pressure was recorded simultaneously to the activity of the cell, in both cases there was no relation between the firing rate and the blood pressure.

One of the criteria for the identification of dopamine cells mentioned in Section C.6, was the analysis of an interval histogram. Examples of typical interval histograms of dopamine cells are shown in Fig. 21.

Stimulation electrodes were implanted during the recording experiment in the fibres leaving the substantia nigra through the medial forebrain bundle, in order to identify the cells by their antidromic spikes. The electrode was implanted either during the acute experiment or at least two weeks before the experiment. The chronic implantation was done with the purpose of testing the animals for intracranial self stimulation, reported to occur with stimulation of these fibres, (75).

Of the 20 animals stimulated in the medial forebrain bundle, in only 5 antidromic spikes were recorded. Three of these spikes collided with spontaneous spikes at 4.0, 4.6, and 5.1 msec respectively therefore probably recorded from cells whose fibres have a conduction velocity of 0.2 m/sec which agrees with the conduction velocity calculated for these fibres of the nigro-striatal pathway, (Chapter I, Section 3.B). Only one cell lasted long enough to study the effect of drugs, (Section D.3.1). The other two spikes antidromically driven were probably recorded from axons, since their duration was less than 2.0 msec and had a latency of 1.0 msec.

D.3 EFFECT OF LESIONS IN THE STRIATO-NIGRAL PATHWAY.

D.3.1 MEDIAL LESIONS.

Cells recorded from 6 rats with a lesion in the ventro-medial area of the crus cerebri, decreased the firing rate significantly after amphetamine and increased significantly after haloperidol. The recording from two of these animals is shown in Fig. 22.

Another rat with a chronic stimulating electrode in the medial forebrain bundle and a ventro-medial lesion of the crus cerebri, had a cell antidromically driven, (Section D.2) in this case, amphetamine produced a decrease in the firing rate statistically significant and haloperidol reversed its effect. Amphetamine administered after haloperidol did not have any effect, (Fig. 23).

D.3.2 COMPLETE LESIONS.

Two rats with all the crus cerebri destroyed also showed a significant reduction in the firing rate after the administration of amphetamine, (Fig. 24).

D.3.3 ACUTE LESIONS.

Three rats form this group. One rat was lesioned 2 hours before the recording. In this case the firing rate of the cell was decreased significantly after amphetamine and increased significantly after haloperidol, (Fig. 25).

In two rats it was possible to hold the cell during the lesioning of the striato-nigral pathway. In both cases amphetamine administration had a significant depressing effect on the frequency of discharge of the cell. In one case, haloperidol injected during the recovery of the amphetamine effect, produced a further significant increase of the firing rate (Fig. 25).

D.4 EFFECT OF THE LESIONS IN OTHER AREAS.

D.4.1 LESION OF SUBTHALAMIC NUCLEUS AND ZONA INCERTA.

One rat that did not display turning behaviour, (Section, C.3) was recorded from the substantia nigra pars compacta. In this case, the firing rate of the cell was also significantly decreased after amphetamine.

The histological analysis indicated that this animal had a lesion extending to the subthalamic nucleus including the zona incerta and Forel's fields, (Fig. 26).

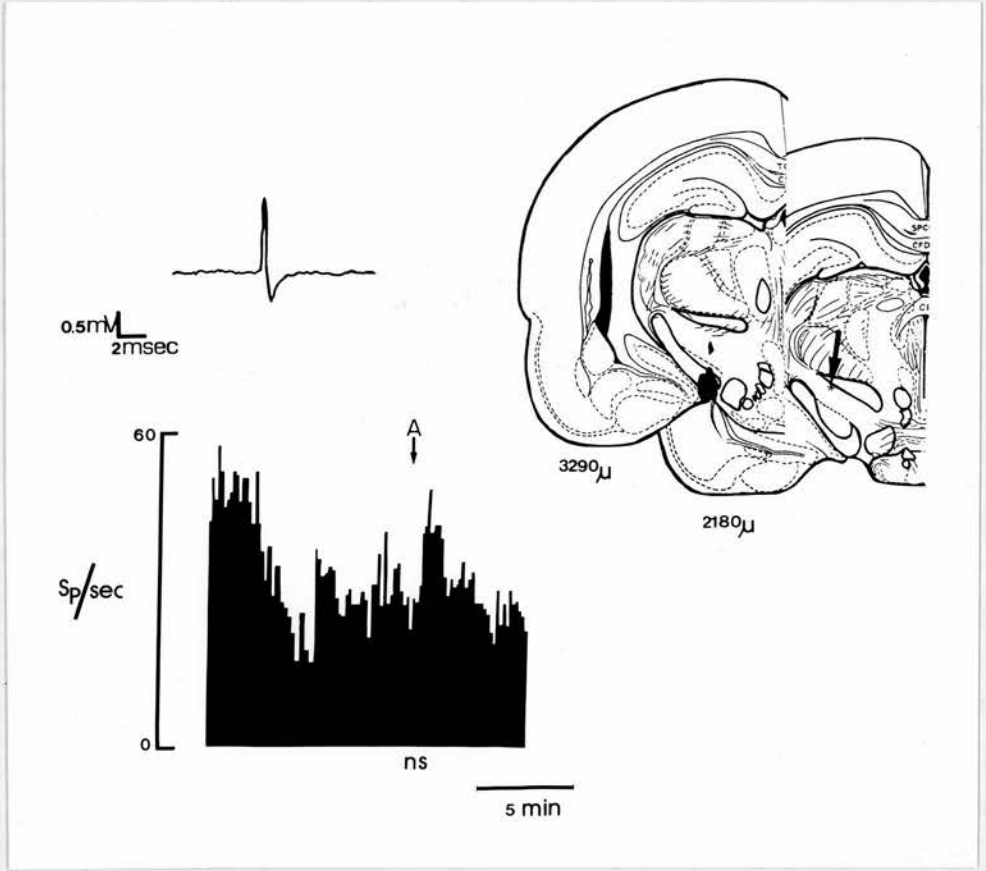


Fig. 20 Effect of amphetamine (A) administration on a cell recorded outside the substantia nigra, (Section D.7).

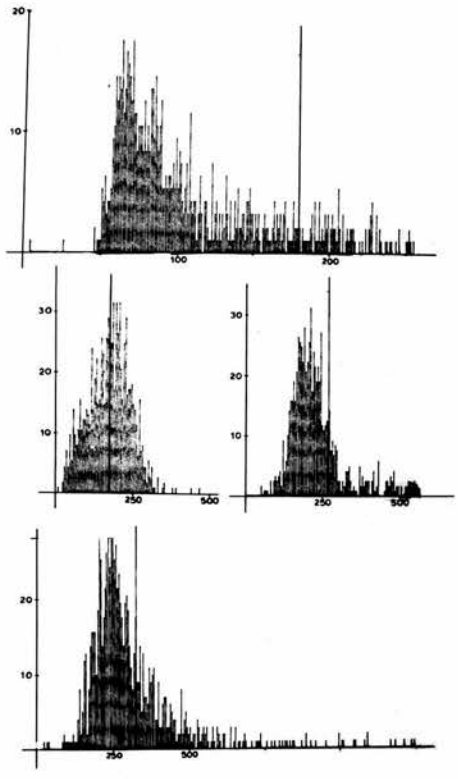


Fig. 21 Interval Histograms obtained from dopamine cells.
(Sections C.6 and D.2).
Vertical Axis: Number of spikes,
Horizontal Axis: Time in milliseconds.

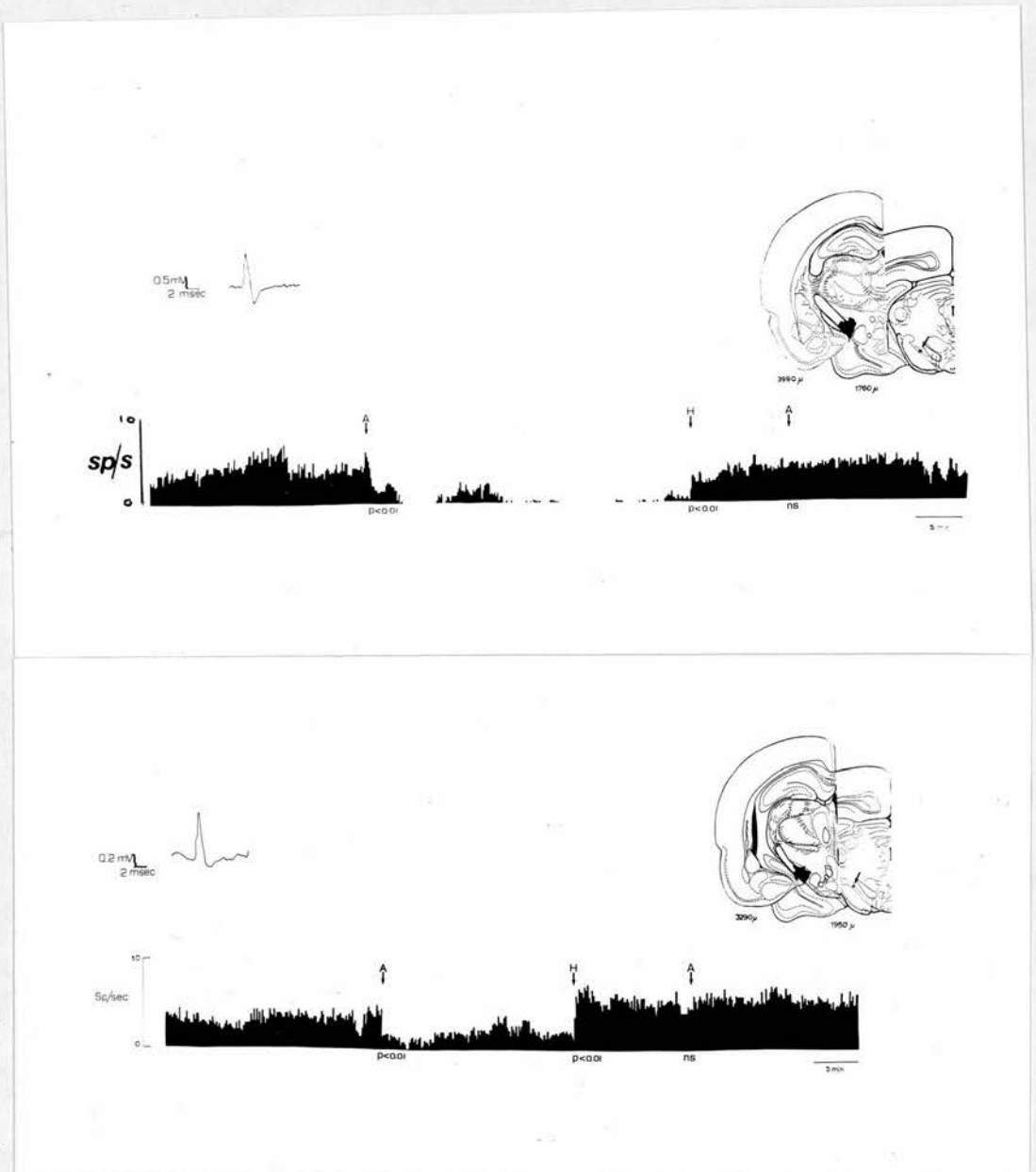


Fig.22 Effect of A LESION IN THE VENTRO-MEDIAL AREA of the crus cerebri, in the response of a dopamine cell to the administration of drugs, Section D.3.1).

Right Corner: Lesioned area (black) and recording site (arrow) plotted on drawings from the Koning and Klippel atlas of the rat brain, (174).

Left Corner: Spike reproduced from a photograph taken during the recording.

Lower Trace: Firing rate of the cell and the effect of: A = Amphetamine (1mg/Kg) and H=Haloperidol (0.1mg/Kg), administered intravenously.

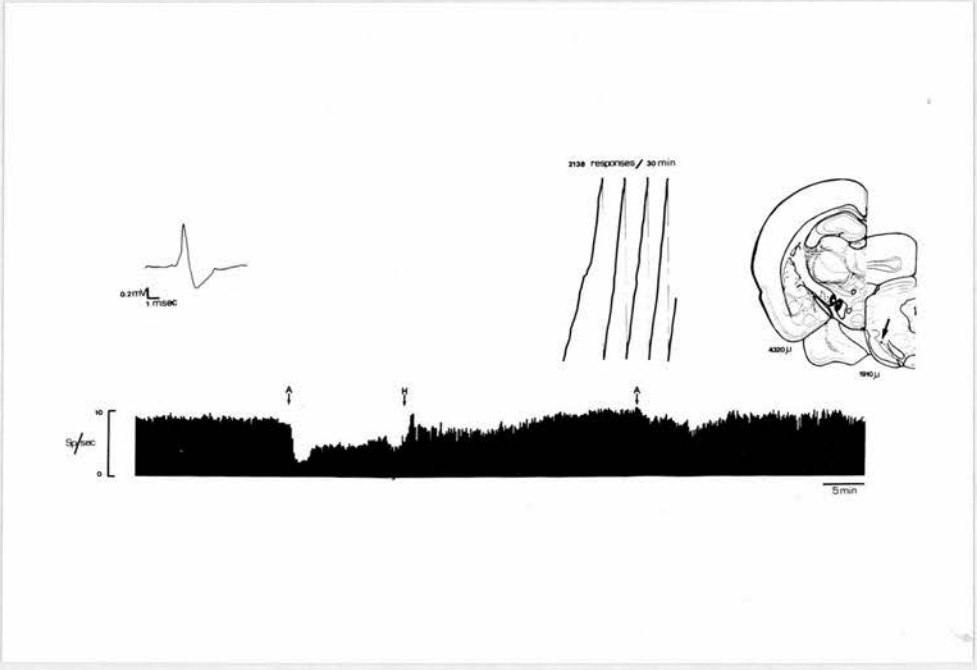


Fig. 23 Effect of a lesion in the ventro-medial area of the crus cerebri on the response of an antidromically driven dopamine cell to the administration of drugs, (Section D.9).

Top Trace: Cummulative record during intracranial self stimulation, with an electrode implanted in the medial forebrain bundle.

Details: Fig 22.

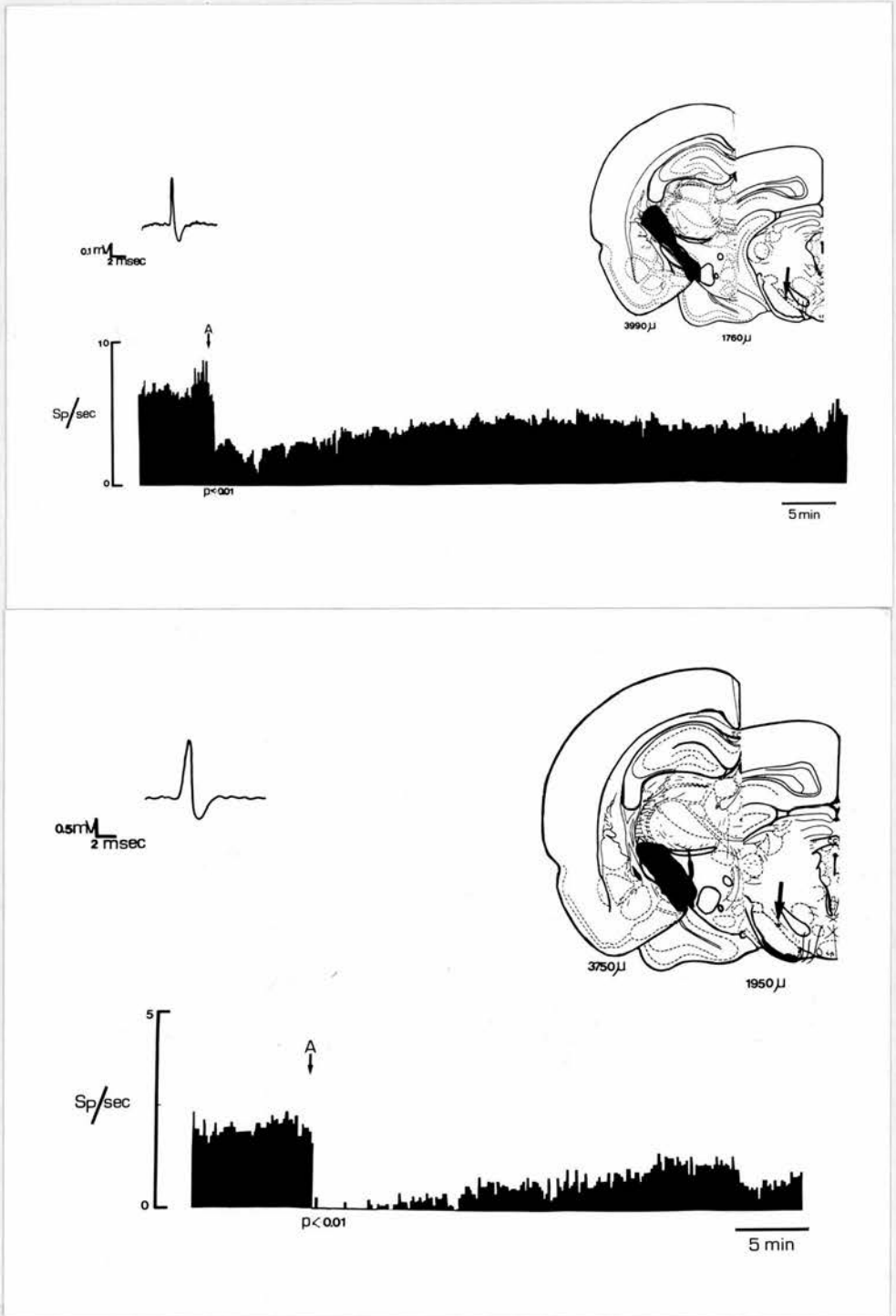
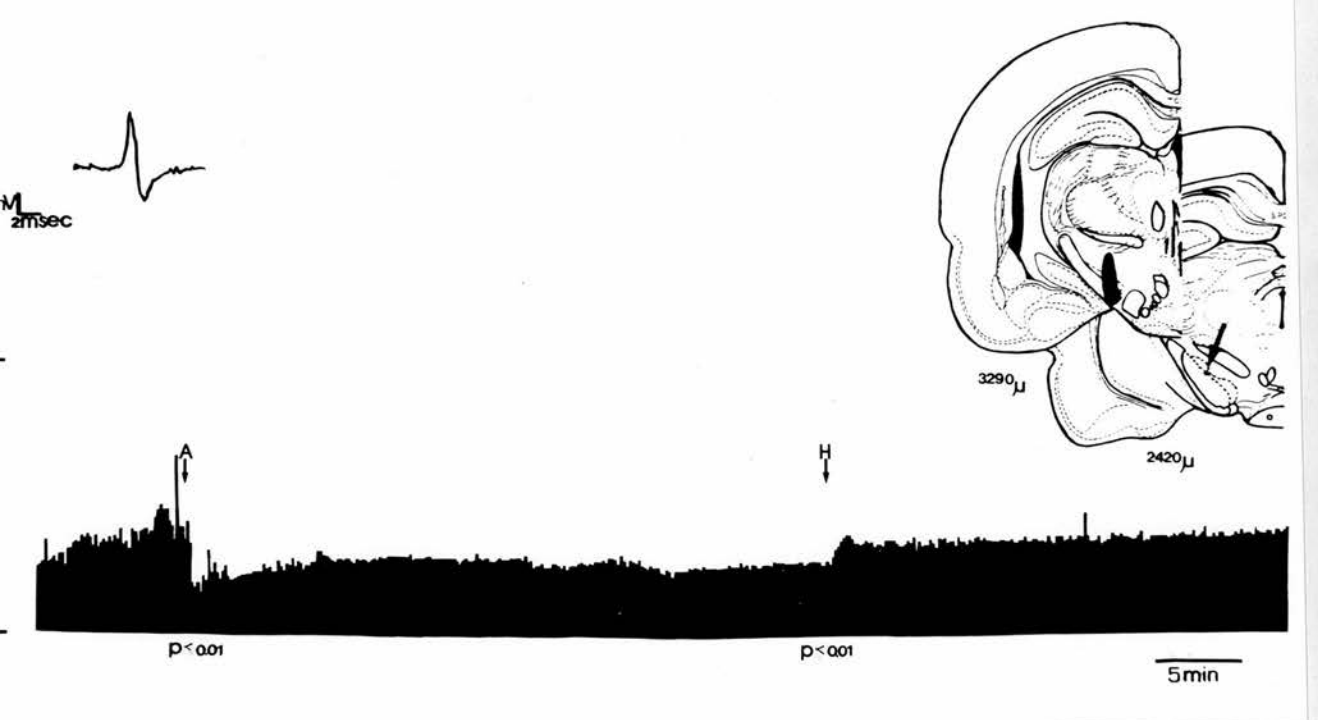
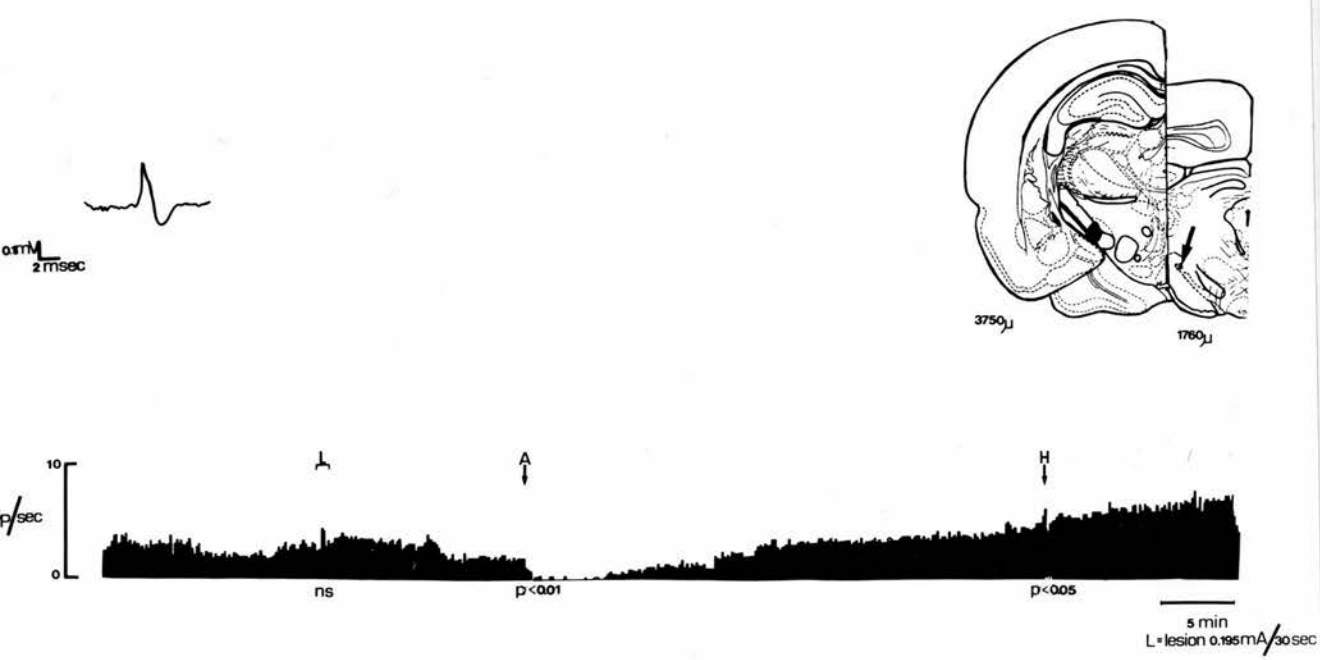
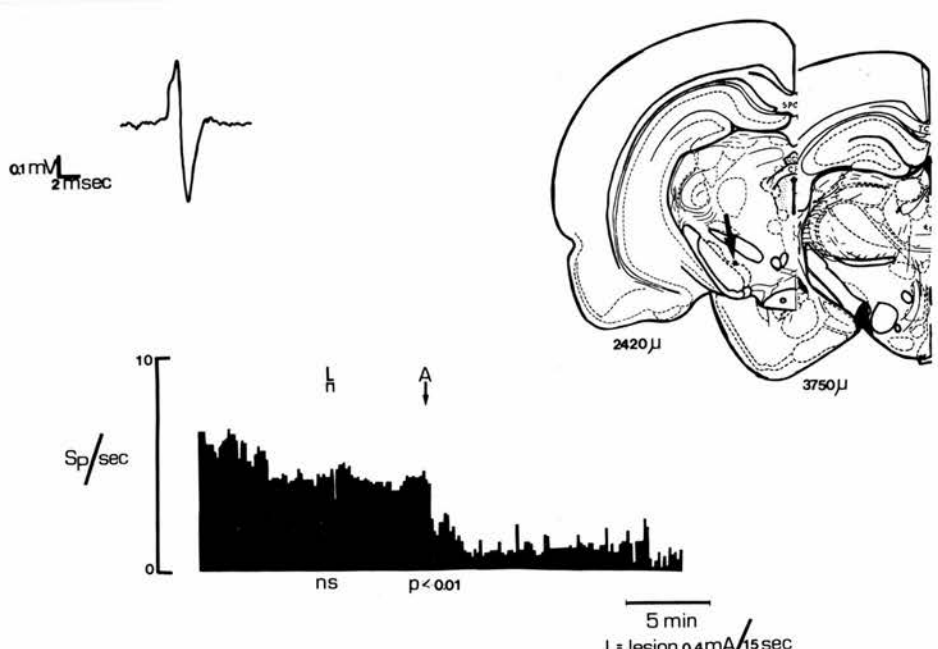


Fig. 24 Effect of a COMPLETE LESION of the crus cerebri, (Section D.3.2) in the response of a dopamine cell to the intravenous administration of drugs.
Details: Fig. 22.


2msec
3290µ
2420µ
A
H
p<0.01
p<0.01
5min


2 msec
3750µ
1760µ
10
p/sec
0
L
A
H
ns
p<0.01
p<0.05
5 min
L= lesion 0.195mA/30sec


2msec
2420µ
3750µ
10
Sp/sec
0
L
A
ns
p<0.01
5 min
L= lesion 0.4mA/15sec

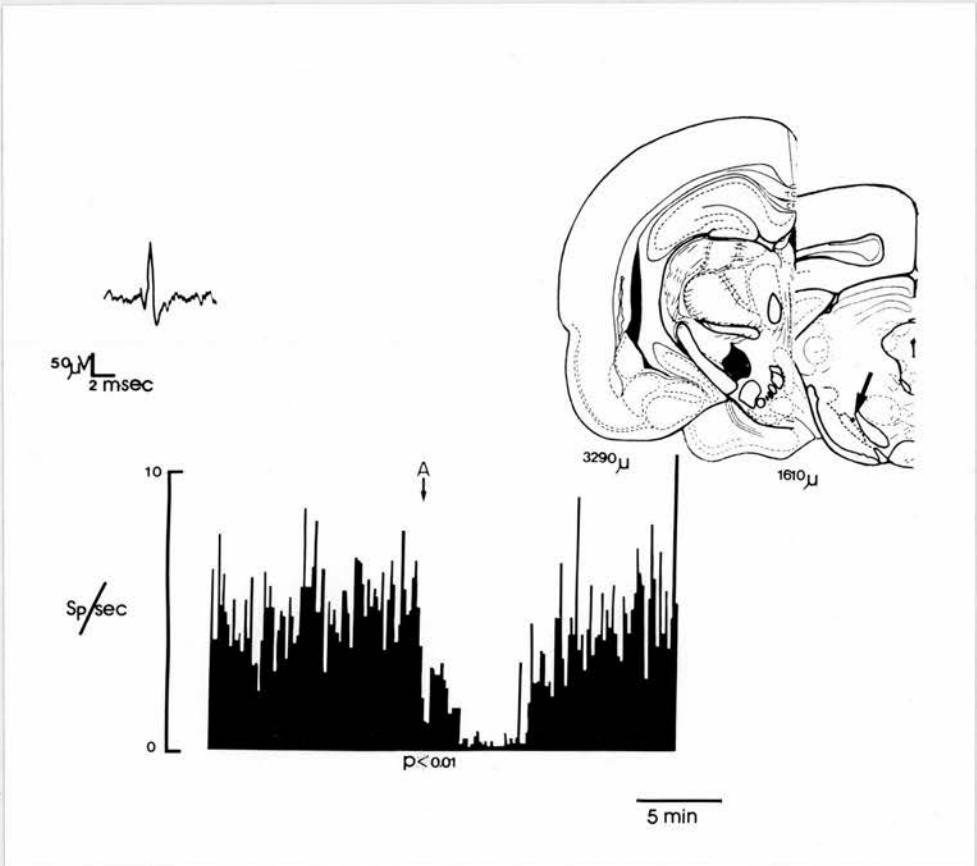


Fig. 26 Effect of a CONTROL LESION above the crus cerebri in the response of a dopamine cell to the intravenous administration of Amphetamine (A), (1.0 mg/Kg), (Section D.4.1). Details: Fig. 22.

D.4.2 PARTIAL LESIONS OF THE SUBSTANTIA NIGRA.

In two occasions the electrode aiming for the crus cerebri was placed too far back, damaging part of the zona compacta and reticularis of the substantia nigra, (Fig. 27.) In both occasions the rats turned more than 100 times/30min.

In one case, neither amphetamine nor haloperidol had a significant effect on the firing rate. In the other, only after two injections of amphetamine, making a total dose of 2 mg/Kg, a significant decrease in the firing rate was observed. Haloperidol administered afterwards did not have a significant effect, (Fig. 28).

To investigate in more detail the consequence of a partial lesion of the substantia nigra pars compacta, injections of 6-OHDA were performed.

D.5 EFFECT OF PARTIAL LESIONS OF THE SUBSTANTIA NIGRA WITH 6-OHDA.

Two rats were successfully partially lesioned. One rat had 23.3% decrease in the concentration of dopamine on the lesioned side, compared to the unlesioned side. In this case, during the recording of the remaining dopamine cells, amphetamine administration did not decrease the firing rate of the cells, but produced a non-significant increase. The other rat only had 12.4% decrease in the concentration of dopamine in the striatum of the lesioned side compared to the intact side. In this case only after a second injection of amphetamine, increasing the total dose to 2 mg/Kg, a significant decrease in the firing rate was observed, but this response only lasted for approximately 150 sec. The injection of haloperidol changed the firing pattern of the cell, but over all the difference was not statistically significant, (Fig.29).

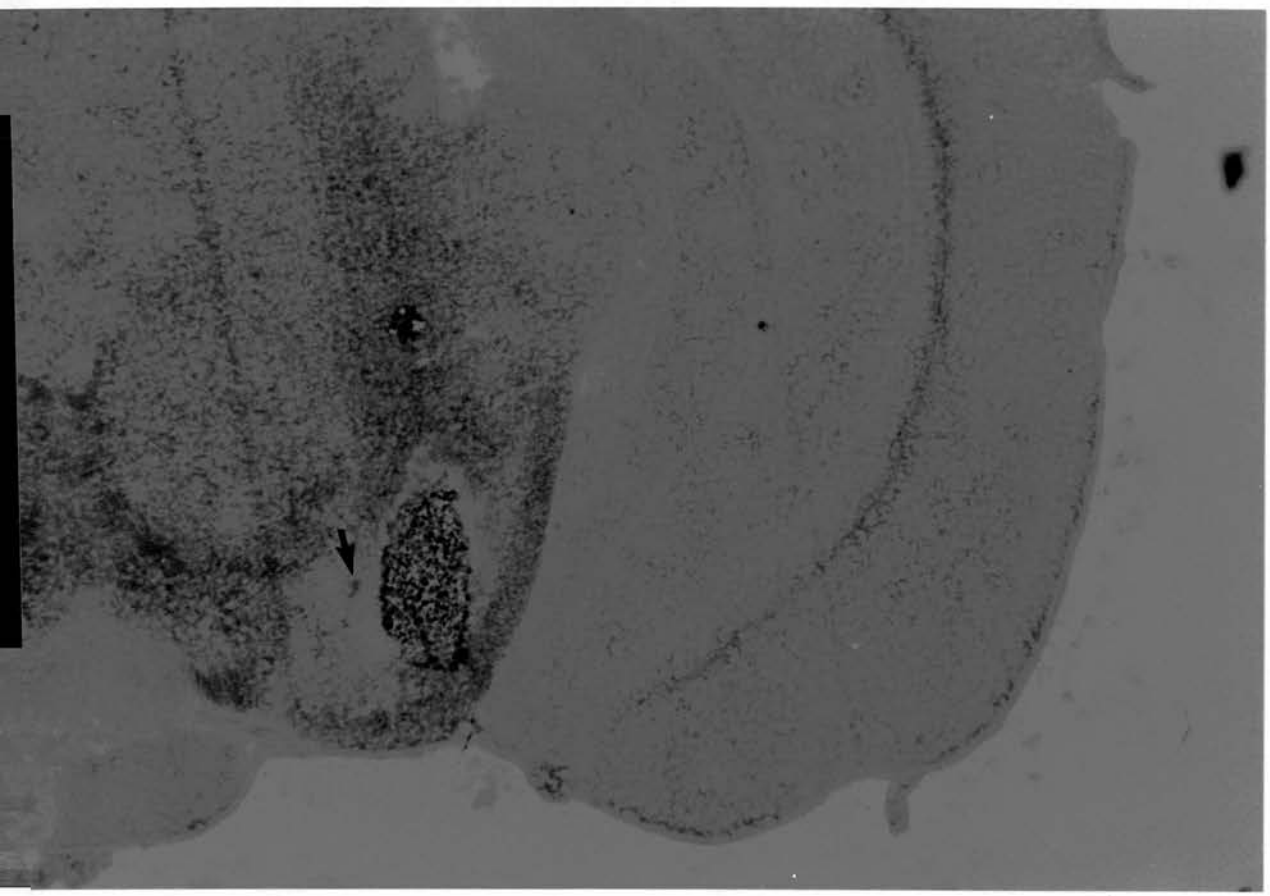
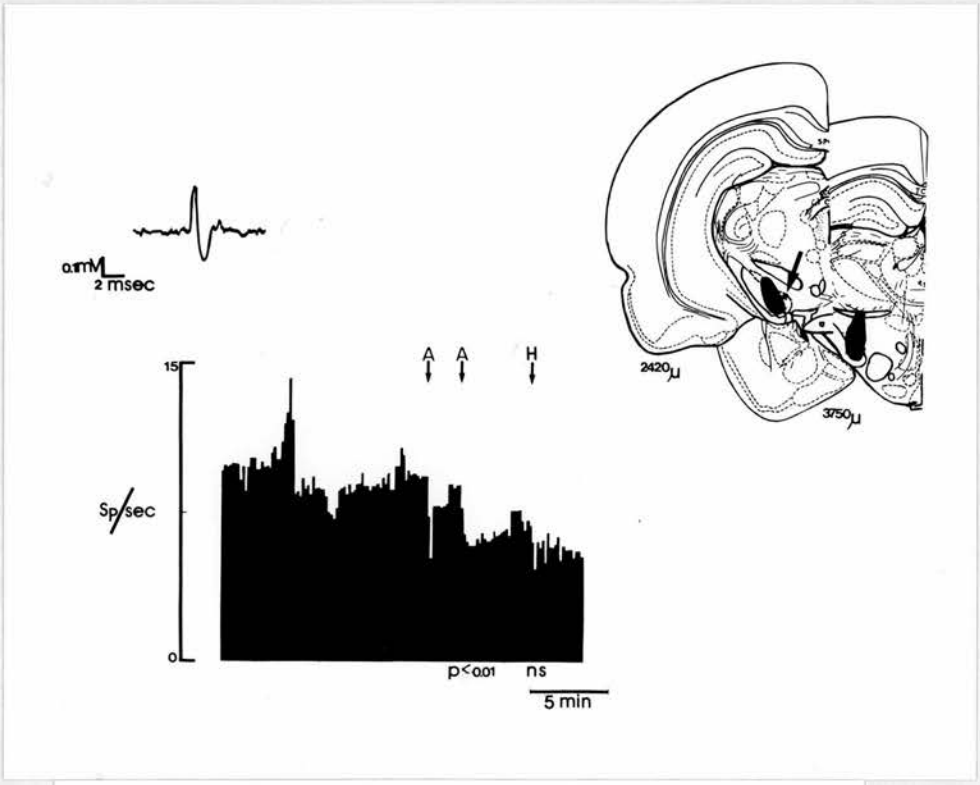


Fig. 27 Lesion including part of the zona compacta and zona reticulata of the substantia nigra, and the dye spot (arrow) indicating the area of recordings (Section D.4.2). Calibration Mark: 1.0mm



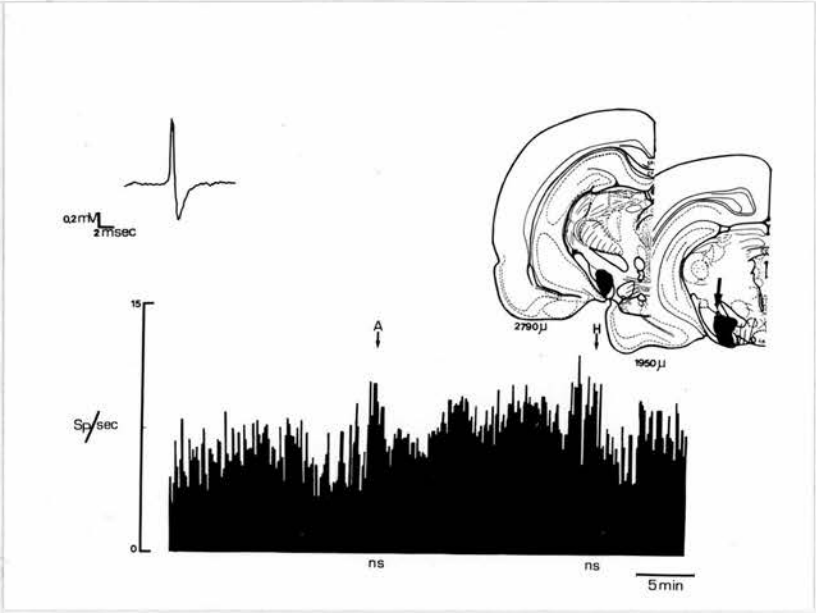


Fig. 28 Effect of a PARTIAL ELECTROLYTIC LESION of the SUBSTANTIA NIGRA on the response of a dopamine cell to the intravenous administration of drugs, (Section D.4.2).

Details: Fig. 22.

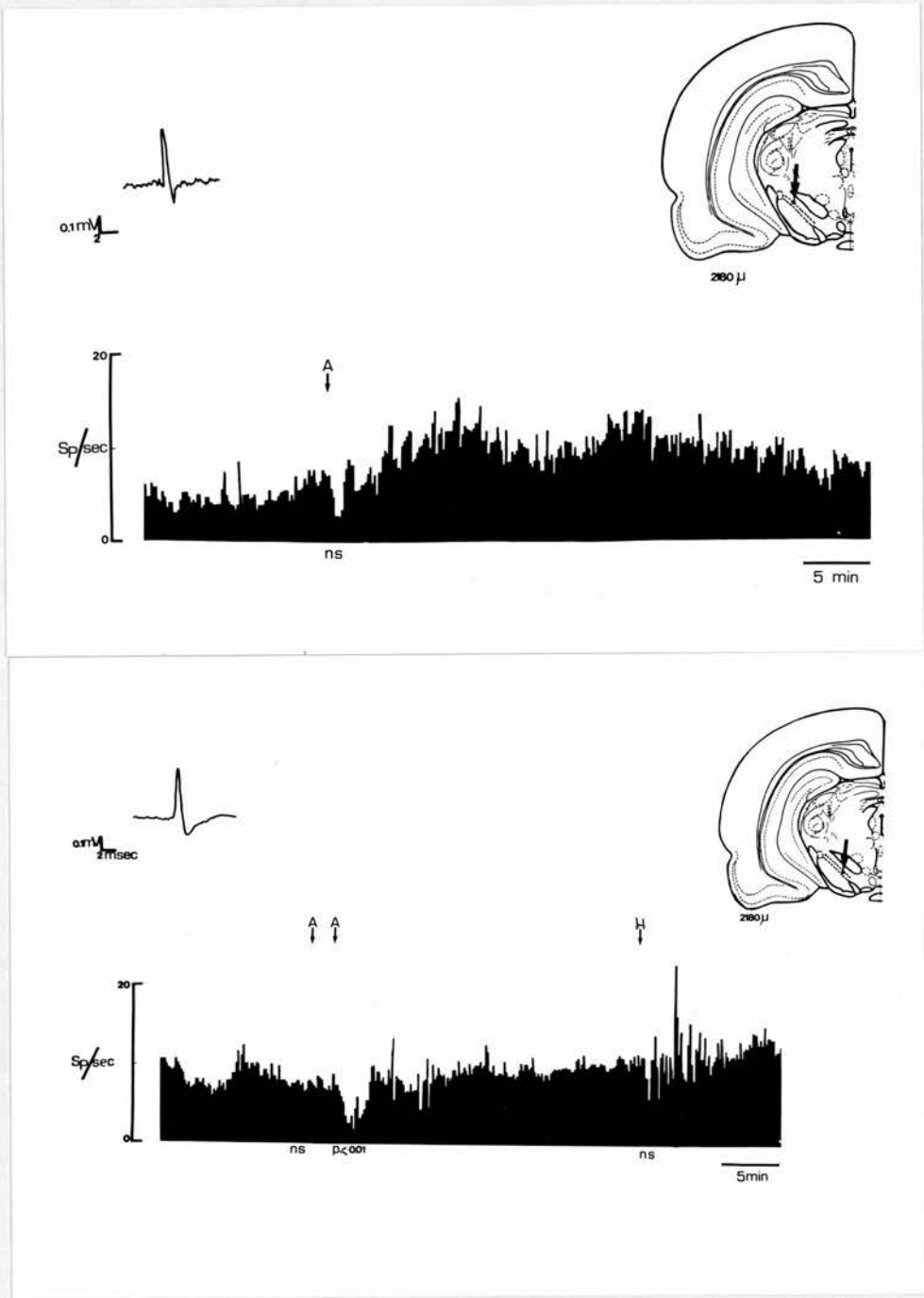


Fig. 29 Effect of a PARTIAL LESION OF DOPAMINE CELLS WITH 6-OHDA on the response of the remaining dopamine cells to the intravenous administration of drugs, (Section D.6). Details: Fig. 22.

D.6 KAINIC ACID LESIONS IN THE STRIATUM.

Six rats were injected with kainic acid in the striatum, but only 3 recovered from the adipsia and aphagia produced by the lesion, (Chapter Iv, Section E). The other 3 died during the first 48 h after the operation. During the recording of cells in the substantia nigra, the administration of amphetamine, produced a significant decrease in the firing rate. In one occasion because the cell died approximately 15 min after the injection, 2 h later, the effect of another injection was studied on another cell. As previously, amphetamine produced a significant decrease in the firing rate of the cell, (Fig. 30). In all cases the administration of haloperidol produced a significant increase in the discharge and blocked the effect of a subsequent injection of amphetamine, (Fig. 30-31).

The histological analysis showed that the lesions included most of the striatum and extended to part of the globus pallidum and the thalamic nuclei, (Fig. 30-31).

D.7 ELECTRICAL STIMULATION OF THE MEDIAL FOREBRAIN BUNDLE.

In nine cells recorded from the substantia nigra pars compacta as indicated by the histological analysis, but not antidromically driven, the effect of the stimulation was studied. A positive-negative potential was averaged with the computer on which a post-stimulus histogram was built up simultaneously. The positive wave was accompanied by inhibition of the firing rate which lasted for 6.6 msec, ($\pm$ 2.3). The positive peak had a latency of 5.8 msec, ($\pm$ 1.0) and the latency of the negative peak was 13.2 msec, ($\pm$ 1.9), (Fig. 32).

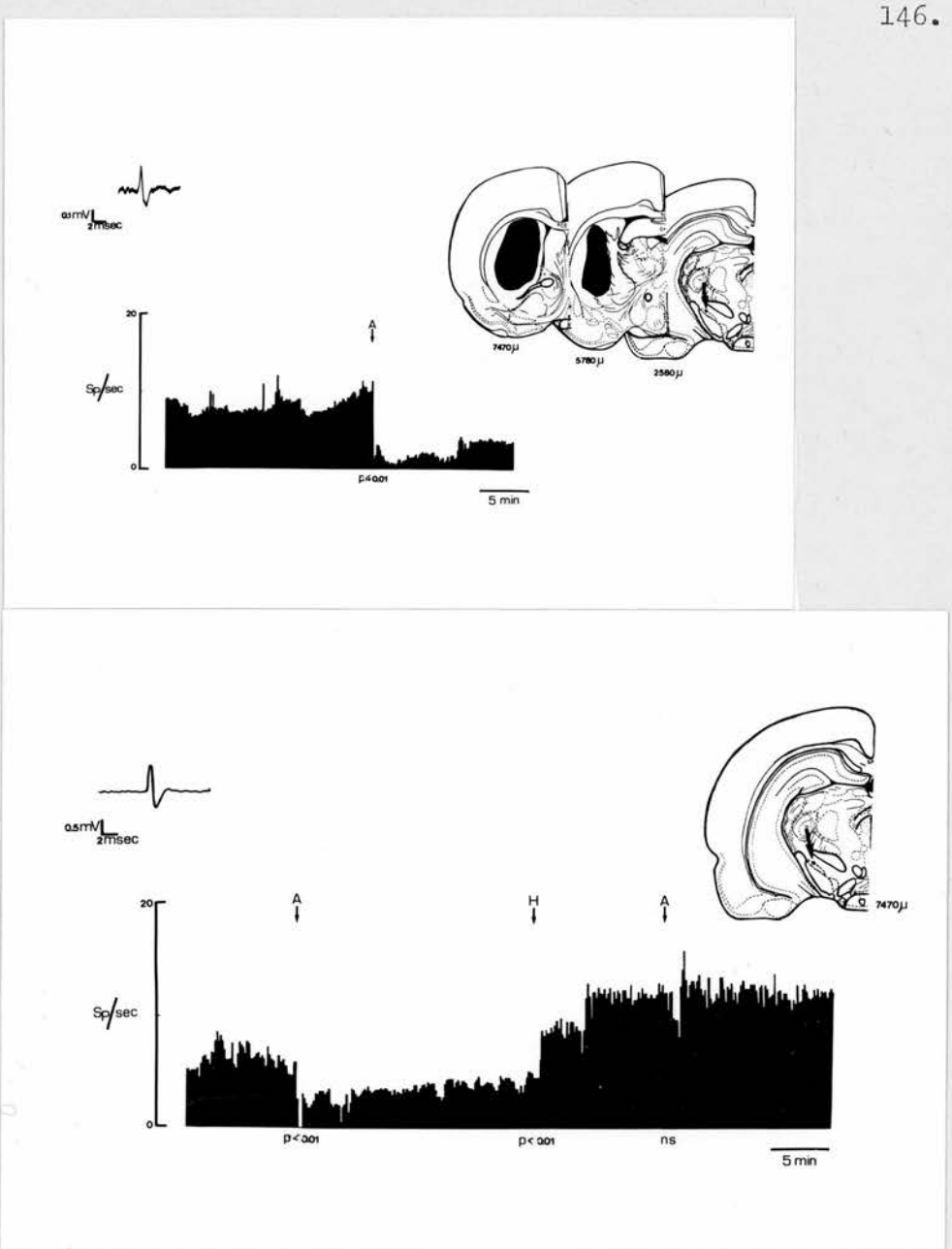


Fig. 30 Destruction of cells in the STRIATUM with KAINIC ACID and its effect on the response of a dopamine cell to the intravenous administration of drugs, (Section D.6).

Top trace: Effect of an injection of A=Amphetamine, (1.0mg/Kg), on the firing rate of the cell.

Lower trace: Effect of Amphetamine (A) and Haloperidol (H), on another cell recorded in the same animal 2 h after the first administration of amphetamine.

Details: Fig. 22.

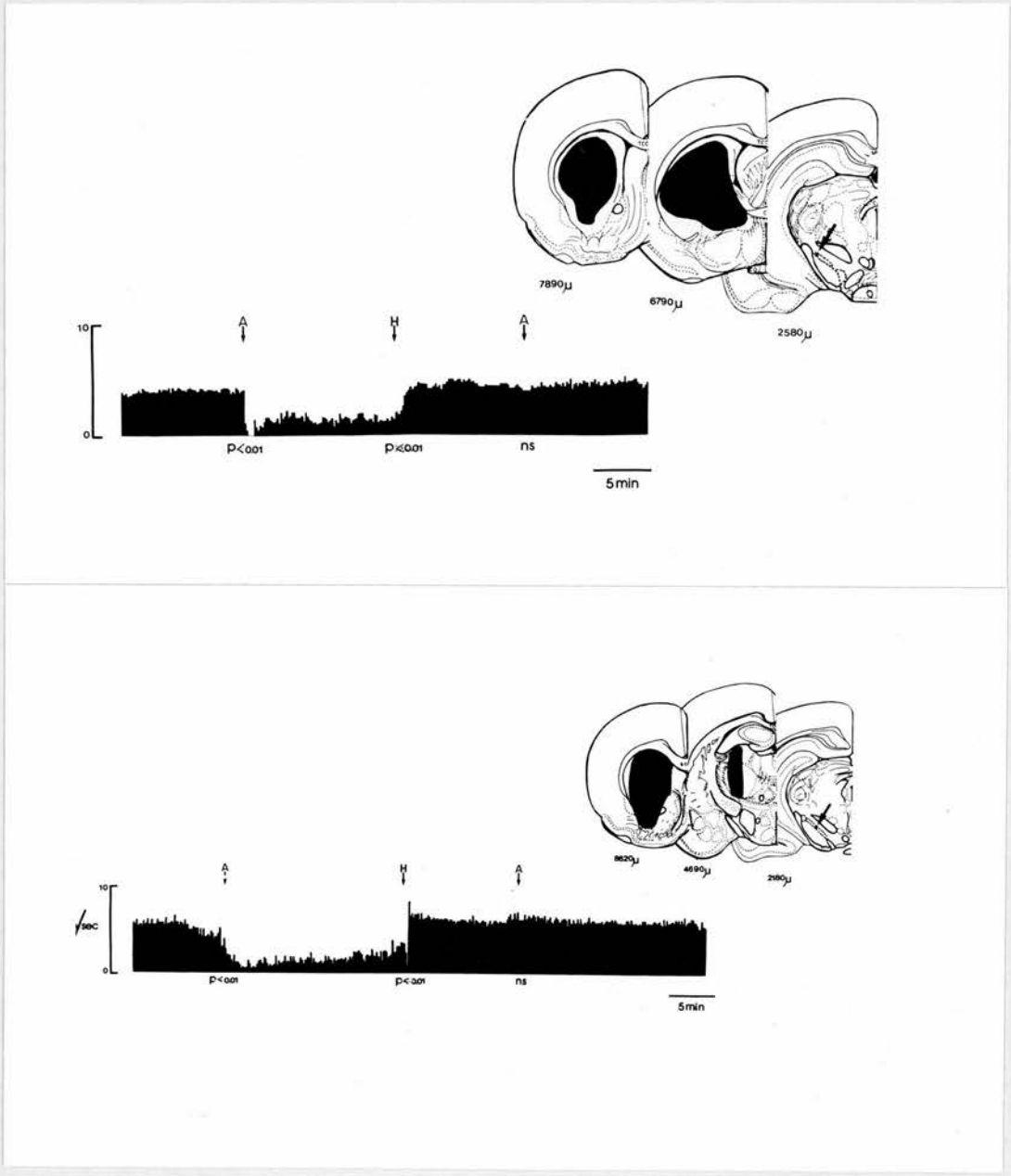


Fig. 31 Destruction of cells in the STRIATUM with KAINIC ACID and its effects on the response of a dopamine cell to the intravenous administration of drugs, (Section D.6). Details: Fig. 22.

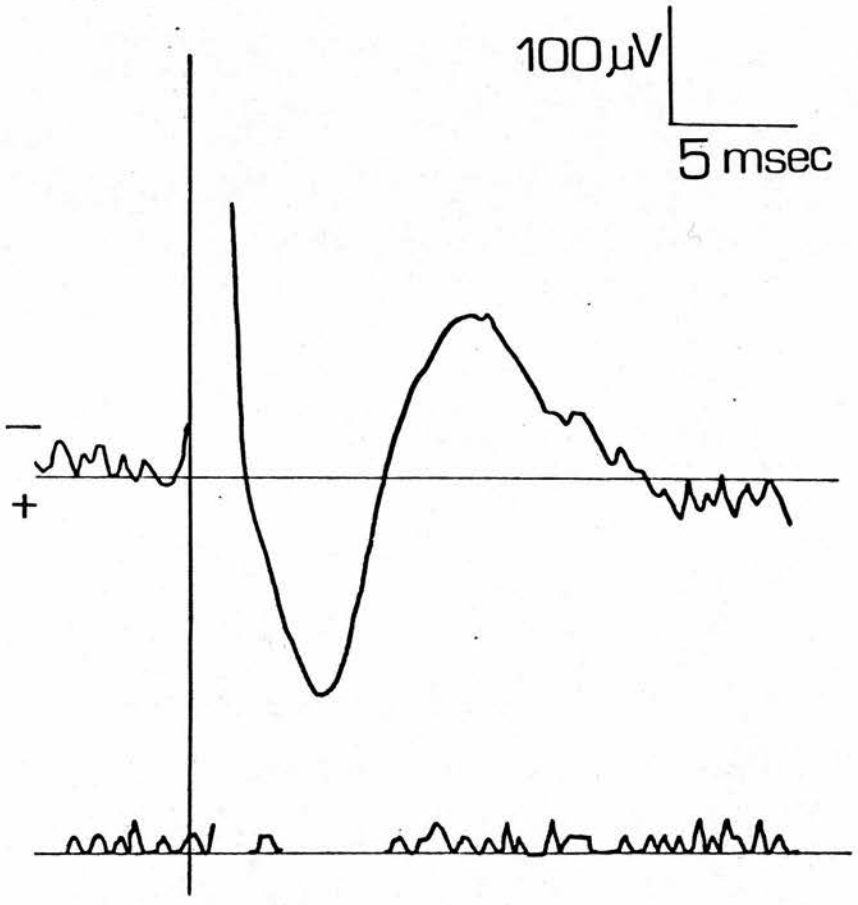


Fig. 32 Response to electrical stimulation with an electrode implanted in the medial forebrain bundle, (Section D.9). Average Field Potential (top trace) and Post Stimulus Histogram (lower trace) taken simultaneously, (100 sweeps).

E. DISCUSSION.

E.1 WHAT IS THE ORIGIN OF THE INHIBITION OF THE FIRING RATE AND THE POSITIVE-NEGATIVE FIELD POTENTIAL EVOKED IN THE SUBSTANTIA NIGRA BY STIMULATION IN THE MEDIAL FOREBRAIN BUNDLE?

A field potential has been recorded in the substantia nigra of rats after stimulation in the striatum, (88). It is believed to be the result of inhibition followed by excitation in the cells, produced by the activity of the striato-nigral pathway. The stimulating current of an electrode in the medial forebrain bundle could spread to fibres of the crus cerebri giving rise to the same response. However, the animals reported in this experiment had a lesion in the crus cerebri which makes this interpretation difficult to sustain. Moreover as demonstrated in the previous Chapter, the striato-nigral pathway does not affect the metabolism of dopamine cells (Section D.4 and E.7) giving evidence against those studies suggesting that the striato-nigral pathway exerts an inhibitory action on these cells. Anatomical evidence is also in agreement with the postulation that this pathway does not control dopamine cells. It has been indicated that the striato-nigral pathway does not end in cells of the substantia nigra pars compacta, but in the pars reticulata, (Chapter I, Sections 1.A and 1.C).

The nature of the inhibition observed in the substantia nigra after stimulation in the striatum must be studied carefully. As already mentioned in Chapter I, Section 3.D most of these reports do not differentiate between the pars compacta and the pars reticulata of the substantia nigra, and it may very well be that the inhibition taken as being produced in the substantia nigra pars compacta is produced in the pars reticulata by activity of the striato-nigral pathway ending in this/

this area but having nothing to do with the dopamine cells. A study differentiating between these areas, has reported inhibition in the substantia nigra pars reticulata in 95% of the cases, (88) supporting this idea, that it is in the pars reticulata where the inhibition occurs in most cases.

If there is an inhibition observed in the substantia nigra pars compacta after stimulation of the striatum, it is probably mediated by other pathways than the striato-nigral one.

The inhibition reported here (Section D.10) could be produced by antidromic stimulation of dopamine fibres which could lead to lateral inhibition by axon collaterals. An argument in favour of this suggestion comes from the difficulty in obtaining antidromically driven cells in the substantia nigra pars compacta (300 and Section D.9.1). Studies on the steps in the production of spikes carried out in motoneurons have shown that when an impulse is generated in a motoneurone there are 3 distinctive components in the rising phase which have been attributed to the successive invasion of different regions in the neuron, (67, 68). This analysis was done mainly studying the effects of the soma membrane potential on the form of an antidromic action potential produced by the stimulation of the ventral roots. The M spike is believed to represent activity in the myelinated region of the axon, the IS spike, is believed to represent the activity of the initial segment, and the SD spike, the activity in the soma and dendrites recorded as a full-sized action potential. Blockage between the IS and SD spikes has been reported after hyperpolarizing the membrane potential, (67, 68, 112). It could be possible to postulate that stimulation of the medial forebrain bundle is inducing dopamine release either/

either from dendrites or axon collaterals into neighbouring cells producing in them a hyperpolarization with the subsequent dissociation between IS and SD components. Recently it has been reported that most cells in the substantia nigra pars compacta show a blockage of the SD spikes, and only the IS spike is recorded making its visualization difficult, (139). This agrees with the idea of an arrangement of cells in the substantia nigra where lateral inhibition can be produced by dendrites or axon collaterals.

It is important to investigate this suggestion further and study the effects of the blockage of dopamine receptors (e.g. by haloperidol) in the antidromic activity and in the inhibition observed in substantia nigra after stimulation with an electrode implanted in the medial forebrain bundle.

Another alternative that must be considered in the production of this field potential and inhibition of the firing rate after stimulation of the medial forebrain bundle is the possibility of exciting cells in the lateral hypothalamus exerting an inhibitory action on dopamine cells. There are reports suggesting the presence of cells in the medial forebrain bundle "path neurons", projecting to the substantia nigra pars compacta, (14). However the anatomical results need to be taken cautiously, since there is no evidence so far that the axons terminate in the substantia nigra, and it is also possible that damage of the fibres around the injection site is the responsible for the results observed.

The origin of the inhibition of the firing rate and the field potential that accompanies it, is therefore not clear. The activity of the striato-nigral pathway is ruled out, leaving as alternatives the activity/

activity of inhibitory cells in the medial forebrain bundle or the production of lateral inhibition by antidromic stimulation of dopamine fibres.

E.2 DOES THE STRIATO-NIGRAL PATHWAY MEDIATE THE RESPONSE OF DOPAMINE CELLS TO AMPHETAMINE?

It was described in Chapter II, that lesions in the ventro-lateral area of the crus cerebri produce the biggest decrease in GAD activity in the substantia nigra. It was also argued that according to anatomical studies, the lesions damaged the striato-nigral and pallido-nigral pathways. The results presented in this Chapter show that lesions in this area do not alter the firing rate of dopamine cells and do not abolish the effect of amphetamine or haloperidol on their firing rate. To eliminate the possibility of remaining fibres travelling in the crus cerebri responsible for the results observed, the whole crus cerebri was lesioned and again the response to drugs was not impaired. The same results were observed with acute lesions and with lesions of cells in the striatum with kainic acid. This indicates that the fibres of the striato-nigral pathway do not mediate the response of dopamine cells to amphetamine, as sustained by the feedback hypothesis, (Chapter I, Section 3.2). There has been a controversy about the effects of amphetamine in the activity of dopamine cells as mentioned in detail in Chapter I, Section 3.F. The results of this experiment point out that amphetamine decreases the firing rate of dopamine cells in the substantia nigra and that its effect is not mediated by the activity of the striato-nigral pathway.

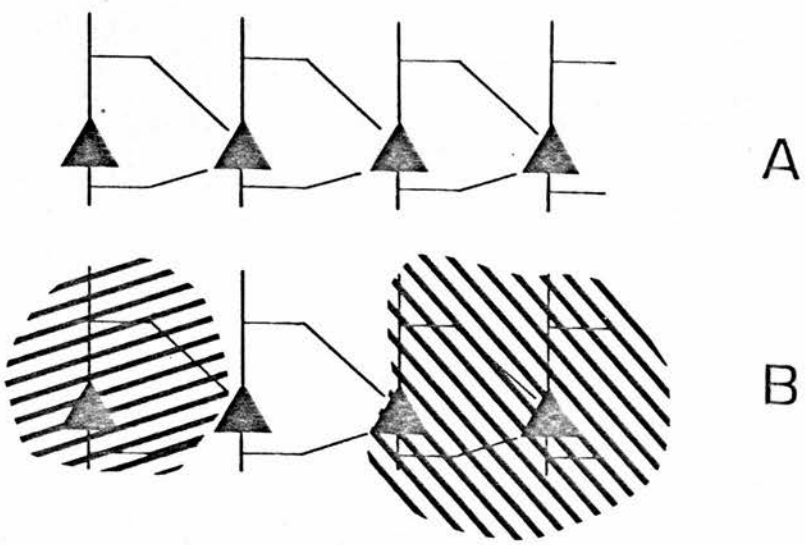


Fig. 33 Schematic representation of dopamine cells in the substantia nigra. A: Lateral Inhibition, B: Partial Lesion, (Section E.2).

E.2.1 POSSIBLE ALTERNATIVES.

Since the lesion of the crus cerebri blocks the participation from the striatum and globus pallidum, the effect of amphetamine could be mediated directly by the drugs inside the substantia nigra or in another structure sending efferents to it. Afferents have been reported from the nucleus accumbens, dorsal raphe nucleus, cortex, habenula and hypothalamus, (43, 272), any of these structures could be affected by amphetamine/

amphetamine resulting in an inhibition of cells in the substantia nigra. However, since dopamine release occurs in the substantia nigra after amphetamine, (Chapter I, Section 2.D) and these cells are inhibited by their own transmitter, (Chapter I, Section E.3), it can be proposed that the response to amphetamine is mediated inside the nucleus. A way of eliminating the participation of other structures and at the same time testing the possibility of an effect locally mediated is through the direct application of amphetamine, into the substantia nigra. This experiment (129) is reported (Chapter I, Section 3.F) and indicates that amphetamine decreases the firing rate of dopamine cells even when directly administered.

It could be proposed that after the administration of amphetamine dendrites or axon collaterals are releasing dopamine and that this is inhibiting their discharge, (Fig.33A). This suggestion is supported by the results of partial lesions of the substantia nigra reported in Sections D.4.2 and D.6. In these cases amphetamine did not inhibit the cells presumably because the neighbouring cells responsible for the inhibition were missing, (Fig.33, B). It also seems likely that previous reports, (130, 42) of lesions which abolish the effect of amphetamine could all have been effective because of the same phenomenon.

E.3 RELEVANCE OF THE EXPERIMENT.

The relevance of the experiments reported in this Chapter lies in the demonstration that the striato-nigral pathway does not exert any influence in the activity of nigral cells as proposed by the feedback hypothesis. According to this hypothesis this pathway exerts an inhibitory/

inhibitory influence on dopamine cells, (Chapter I, Section 3). This suggestion was supported by the activity of substantia nigra cells after stimulation of the striatum, however as it has been discussed the evidence that in fact the cells were from the pars compacta is very poor, the histological analysis is not shown in many occasions (305, 103, 108) and no criteria to differentiate between cells in one area and the other were applied (88, 204, 114, 113).

Further support to the feedback hypothesis came from experiments indicating that the firing rate of dopamine cells was not affected after systemic administration of amphetamine if the fibres of the striato-nigral pathway were damaged either by a hemisection (40) or a lesion of the crus cerebri (42) immediately before the recording. This was taken as evidence to say that amphetamine was acting in the terminals of dopamine fibres in the striatum releasing dopamine there which in turn activated the striato-nigral pathway to produce inhibition of dopamine cell bodies, (39, 132, Chapter I, Section E.3.2).

The experiment reported here show that a lesion of the crus cerebri does not alter the response of the cell to amphetamine and supports the experiments showing an inhibition of dopamine cells after administration of amphetamine, (129, 130, Chapter I, Section 3.F).

The only case when amphetamine did not inhibit the firing rate of cell was when a partial lesion of the substantia nigra was made, (Sections D.4.2 and D.5). It is important to mention that the experiments reported elsewhere involving acute lesions, (297, 42) which gave evidence in favour of the feedback hypothesis, do not report any histological analysis, therefore making it difficult to know if the lack of effect by amphetamine was given a similar partial lesion/

lesion of substantia nigral cells as the reported here. In fact the only experiment which mentions histological results, (130), reports that their lesion extended to the substantia nigra. Therefore it can be concluded, by the experiment reported in this Chapter, that it is not the lesion of the striato-nigral pathway the one that affects the response to amphetamine, but a lesion of substantia nigral cells, which probably affects a system of lateral inhibition.

SUMMARY.

I - Stimulation of fibres in the medial forebrain bundle inhibited the firing rate of dopamine cells and evoked a positive-negative field potential. Stimulation of this area produced antidromic spikes in 5 animals of the 17 tested.

II - The firing rate of dopamine cell decreased significantly after the intravenous administration of amphetamine in the following groups of rats:

A - Control.

B - Chronically lesioned in: striatum, ventro-medial area of the crus cerebri and the whole crus cerebri.

C - Acutely lesioned in the ventro-medial area of the crus cerebri.

Haloperidol reversed the response to amphetamine and blocked the effect of a subsequent administration of amphetamine, in these groups.

III- The following lesions were successful in abolishing the effect produced by the administration of amphetamine, on the firing rate of a dopamine cell:

A/

A - Partial electrolytic lesions of the substantia nigra.

B - Partial lesions of dopamine cells with 6-OHDA.

It is concluded that the striato-nigral pathway does not mediate the decrease in the firing rate of dopamine cells observed after the intravenous administration of amphetamine.

CHAPTER IV

BEHAVIOURAL ANALYSIS OF RATS WITH A LESION IN THE STRIATO-NIGRAL PATHWAY.

CHAPTER IV

BEHAVIOURAL ANALYSIS OF RATS WITH A LESION IN THE STRIATO-NIGRAL PATHWAY.

A. INTRODUCTION.

A lesion of dopamine cells by intranigral injection of 6-hydroxydopamine, (6-OHDA), on one side of the brain, produces a degeneration of the nigro-striatal pathway and causes rats to deviate their posture towards the lesioned side, (287). In these animals, dopamine agonists, (e.g. apomorphine or amphetamine) induce a dose dependent rotation, (287).

The involvement of the nigro-striatal pathway in turning behaviour has been supported by studies involving electrical stimulation of the pathway. During continuous electrical stimulation, the rats circle ds the unstimulated side and this behaviour is blocked by haloperidol, (13).

Drug interaction studies confirmed the involvement of dopaminergic mechanisms in rotation. Inhibition of tyrosine hydroxylase alpha-methyl-para-tyrosine, (AMPT), blocks the effect of amphetamine, but not that of apomorphine,(116). Haloperidol antagonizes both amphetamine and apomorphine effects, (287).

Stimulation of the caudate nucleus of cats, evokes movement of the head and turning behaviour opp. site to the stimulated site, (184). The same behaviour pattern can be reproduced with an injection of dopamine into the nucleus, which also facilitates the response produced by electrical stimulation, (66). Haloperidol or AMPT directly into the nucleus suppresses the effects of electrical stimulation/

stimulation, suggesting that the activity of the nucleus altered by changes in dopamine concentration must be involved in the display of turning behaviour.

Electrolytic lesions in the striatum and globus pallidum in rats or in the caudate nucleus and interpeduncular nucleus in cats, (78, 79) produces spontaneous turning towards the lesioned side which is increased by intraperitoneal injections of L-Dopa, apomorphine or amphetamine. These drugs presumably act on the dopamine receptors of cells in the intact side of the brain.

On the other hand, the striatum seems to participate in other behaviours like passive avoidance, (109, 240), go no-go tasks, (172) and lever pressing, (257), since its lesion or stimulation alters the performance of the task.

The activity of the striatum is therefore responsable, at least in part, for the expression of these motor behaviours. Since a major output from the striatum is through the striato-nigral pathway ending in the substantia nigra pars reticulata, (Chapter I, Section 1.A), and the results presented in Chapters II and III indicate that it is not part of a feedback system to control dopamine metabolism, it was decided to study the participation of the striato-nigral pathway in motor behaviour, in order to clarify its possible function.

In this Chapter changes in paw preference and the amount of turning behaviour after dopamine agonists were examined after unilateral electrolytic lesions in the ventro-medial area of the crus cerebri. The results presented, indicate that the lesions induce a dose dependent turning towards the lesioned side, decreases the number of turns of a 6-OHDA lesioned animal by 75.5% and reverses the preference of the paw/

paw used for lever pressing, after a lesion contralateral to the preferred paw. A possible cicrcuit involved in the generation of these motor behaviours is discussed.

B. PROCEDURE.

There were 3 groups of rats with lesions in the striato-nigral pathway:

1 - The animals were lesioned, allowed to recover for a week and tested for turning behaviour.

2 - The rats were kept in reverse day light conditions and received 30 min sessions of behavioural training in a Skinner box, every day. Once they learned to press a lever to obtain food, their paw preference was observed during 5 sessions. Then the rats were lesioned on the contralateral side to the preferred paw, allowed to recover for a week and tested for lever pressing for another 5 sessions. Then they were tested for turning behaviour.

3 - The animals received first a unilateral injection of 6-OHDA into the substantia nigra on one side of the brain, were allowed to recover for a week and tested for turning behaviour. Then lesioned again, this time in the ventro-medial area of the crus cerebri ipsilateral to the previous lesion, allowed to recover for a week and re-tested for turning behaviour.

Once the behavioural analysis was done, the rats were sacrificed, the brain removed for dissection and the biochemical determinations and the histological analysis performed.

. METHODS.

The procedure for stereotaxic surgery, electrolytic lesions and

ost/

post-operative observations has been described in Chapter II, Sections C.1, C.1.1 and C.2. For the lesions in the ventro-media area of the crus cerebri the co-ordinates used were the same as described in Chapter II, Section C.1.1.

When a sham operation was performed, the animal was anaesthetized and placed in the stereotaxic frame, the skull was exposed and a hole was bured in the same place as the lesioned rats. Then the skin was sutured and the animal was allowed to recover as the lesioned animals. For the 6-OHDA injections, the co-ordinates used were the same as described in Chapter III, Section C.2, but since this time a complete lesion was desired the concentration of 6-OHDA was 2.0 mg/ml; the volume injected was 4.0 ul.

The procedure for the biochemical determinations of GAD activity and dopamine concentrations have been described in Chapter II, Section C.3.5 and C.3.2 respectively.

C.1 BEHAVIOURAL OBSERVATIONS.

The animals were housed in groups of 3 per cage and kept in a cycle of 12 hours reverse day light conditions.

C.1.1 TURNING BEHAVIOUR.

The test was carried out in a spherically shaped plastic bowl of 390 sq. cm. The number of complete circles was counted by observation during 30 min. The counting started 1 min after the injection of drugs. Any other behaviour was also noted.

C.1.2 LEVER PRESSING.

The animals were deprived of food for 48 hr before the first day of training, thereafter were fed once a day after the training session allowing/

allowing food ad libitum for 1 hr.

The first training session lasted for 45 min, during which the association lever press-food was established. Then the sessions lasted 30 min. The paw preference was determined observing every animal and counting the number of times it pressed with one, the other or both paws.

C.2 DRUGS ADMINISTERED.

DRUG	DOSE	CONCENTRATION
For crus cerebri lesioned animals:		
D-Amphetamine	2 mg/kg	2 mg/ml
Apomorphine	2 mg/kg	2 mg/ml
For 6-OHDA lesioned animals:		
Apomorphine	0.2 mg/kg	0.2 mg/ml

All drugs were administered intraperitoneally.

D. RESULTS.

D.1 HISTOLOGICAL ANALYSIS.

The rats that turned consistently towards the lesioned side more than 100 times/30 min were found to have a lesion in the ventro-medial area of the crus cerebri similar to the one illustrated in Chapter II, Fig. 7.

The animals which did not display turning behaviour, had lesions in other places around the crus cerebri like the hippocampus, amygdala, zona incerta and Forel's fields, (Chapter II, Section D.1.1).

D.2 TURNING BEHAVIOUR.

When the animals were injected with amphetamine or apomorphine the posture which by general examination did not differ from control rats, deviated towards the lesioned side. The tail curved towards the concave torax and the rat walked in closed circles. Sniffing occurred and there was little grooming activity. Turning started approximately 3 min after the injection of the drugs and lasted for approximately 30 min after apomorphine and 1 hr after amphetamine administration. Both drugs induced turning towards the lesioned side.

Six rats with a lesion in the ventro-medial area of the crus cerebri turned 192.5 times/30 min ($\pm$ 77.4) after amphetamine administration. Ten rats with a similar lesion turned 184.6 times/30 min ($\pm$ 73.4) after apomorphine administration, (Fig. 34).

When the extent of the lesion was compared to the number of turns after the apomorphine administration (Chapter II, Fig. 10), it was observed that rats with lateral lesions turned a smaller number of times than rats which had the whole crus cerebri destroyed, although the difference was not statistically significant.

A dose-response curve to apomorphine was constructed with 7 rats, (Fig. 35), which shows that turning is dose dependent.

D.3 PAW PREFERENCE.

The preferred paw was determined for each rat before the lesion. Two rats pressed more times with the right paw and the other 4 with the left. After a lesion contralateral to the preferred paw all rats changed their preference and started to use more the extremity ipsilateral to the lesioned side. The control animal kept using the same/

same paw after a sham operation, (Table XI and Fig. 36).

The side on which the lesion changed effectively the paw preference was determined on a trial group of 5 rats lesioned in the left side of the brain independently of their side preference. Only two rats with a lesion contralateral to the preferred paw changed their preference.

The histological analysis showed that all these rats had a lesion in the ventro-medial area of the crus cerebri, although not all of the same magnitude. This was confirmed by the biochemical analysis which showed that GAD activity was decreased in the substantia nigra ipsilateral to the lesioned side, in a range from 16.5% to 53.7%. The concentration of dopamine in the striatum of the lesioned side did not differ significantly from its concentration on the unlesioned side, (Table XII).

D.4 6-OHDA PLUS CRUS CEREBRI LESIONS.

The number of turns after a crus cerebri lesion in a 6-OHDA injected animal was significantly reduced compared to the number of turns after the 6-OHDA lesion alone, (Table XIII). The biochemical analysis showed that dopamine in the striatum ipsilateral to the 6-OHDA injection, was significantly reduced, and GAD activity was also significantly reduced in the substantia nigra of the same side, (Table XIII, and Fig. 37).

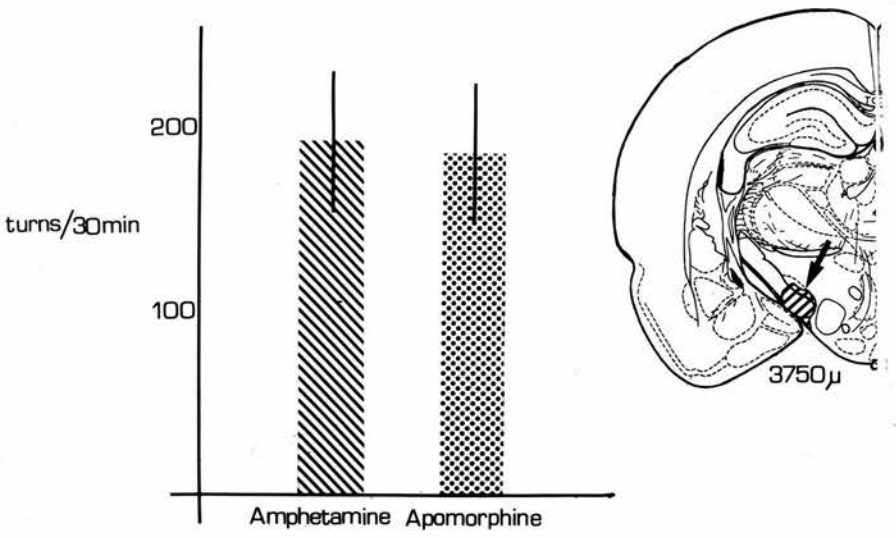


Fig. 34 Turning behaviour induced after amphetamine (2mg/kg) and apomorphine (2mg/kg) towards the lesioned side.
Representative lesion (arrow) plotted on a diagram from the Koning and Klippel atlas of the rat brain (174).
Bars = standard deviation of the mean (Section D.2)

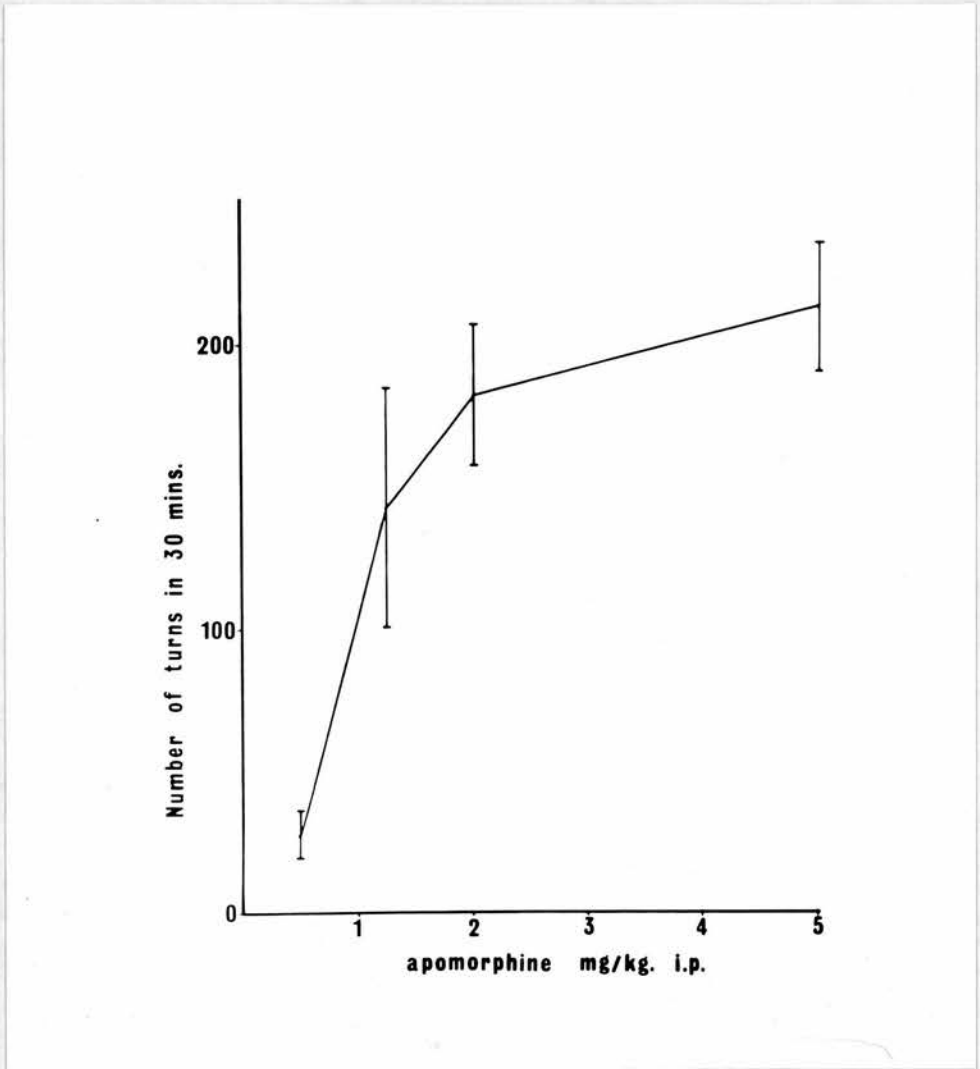


Fig. 35 Dose response curve to apomorphine, in rats with lesions in the ventro-medial area of the crus cerebri.

Bars = Standard Deviation of the mean.

(Section D.2)

TABLE XI

PAW PREFERENCE.

Mean of the difference of lever presses (preferred - non preferred paw) in 5 sessions.

	PREFERRED PAW		EXPERIMENTAL GROUP				CONTROL
BEFORE LESION	Right	$\bar{X}$	5.4	16.4			
		S.E.M.	1.9	2.1			
	Left	$\bar{X}$			68.4	42.8	40.8 69.8
		S.E.M.			14.1	12.2	12.0 16.5
	Right	$\bar{X}$			39.8	69.6	25.6
		S.E.M.			30.7	10.1	10.6
AFTER LESION	Left	$\bar{X}$	100.4	58.2			151.0
		S.E.M.	14.1	4.1			6.7
Number of turns/30 min			93	120	114	139	154

TABLE XII

PAW PREFERENCE

		EXPERIMENTAL GROUP					CONTROL
Dopamine in Striatum	Lesioned side	9.701	8.133	10.986	9.739	11.095	10.780
	Unlesioned side	9.635	8.886	9.600	8.506	9.750	9.802
AD ctivity n igra	Lesioned side	503.2	428.5	342.3	431.7	526.5	587.2
	Unlesioned side	602.0	588.3	652.1	932.7	691.3	518.4
	% Decrease	16.5	27.2	47.5	53.7	23.8	

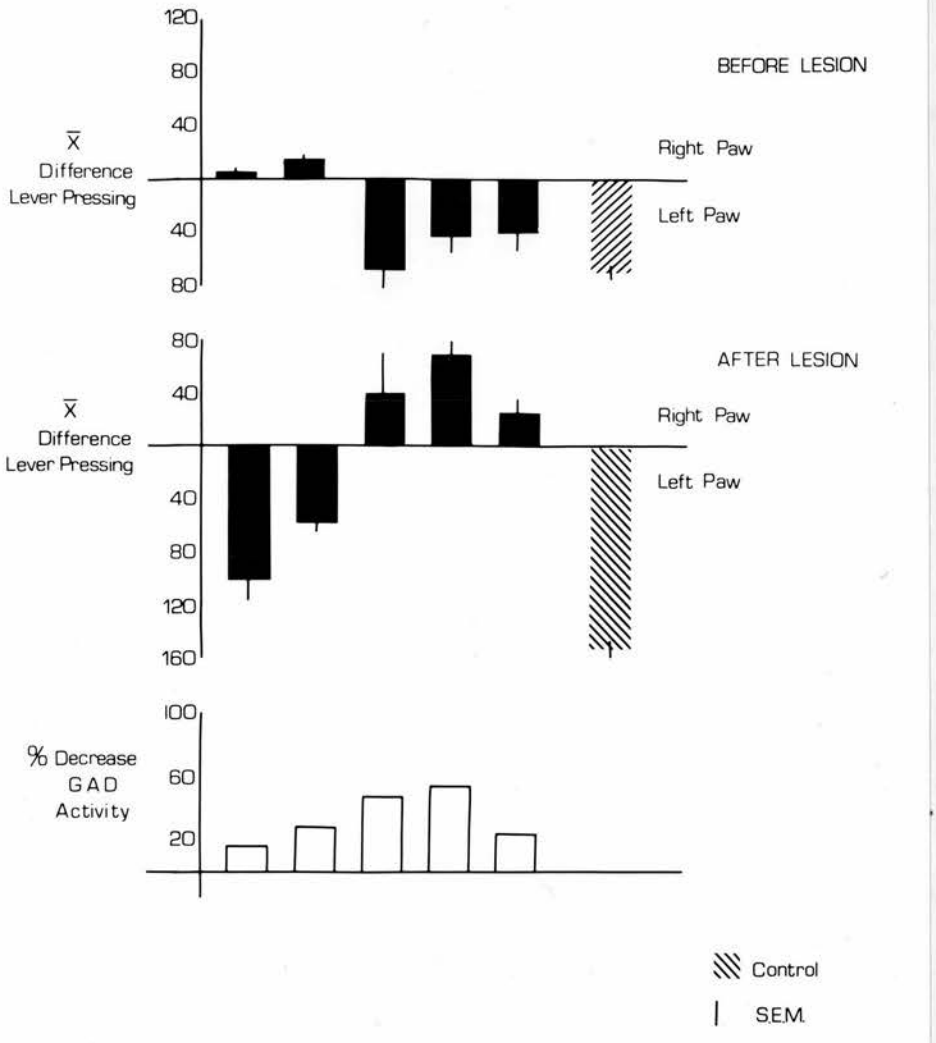


Fig.36 Top graphs: Paw preference during 5 sessions, plotted for each rat before and after a lesion in the striato-nigral pathway.

Lower graph: Percentage decrease in GAD activity compared to the unlesioned side for each lesioned animal.

(Section D.3).

TABLE XIII

6-OHDA PLUS CRUS CEREBRI LESIONS

Rat Number	NUMBER OF TURNS AFTER					
	6-OHDA LESION	6-OHDA PLUS CRUS CEREBRI LESION	DA STRIATUM		GAD NIGRA	
			L	C	L	C
1	365	29	0.117	10.500	384.1	1015.1
2	261	24	0.051	5.269	302.2	674.7
3	246	89	0.063	9.495	169.0	263.2
4	272	22	0.150	7.513	136.6	188.3
5	310	237	1.507	5.438	390.5	483.0
6	325	127	0.113	8.730	335.5	521.4
7	267	9	0.149	9.694	241.2	261.0
8	127	2	8.917	8.882	244.4	277.4
9	98	34	0.164	8.867	95.4	201.0
0	104	9	6.847	8.930	597.3	824.6
	237.5	58.2	1.012	8.331	299.0	461.5
.E.M.	30.1	23.5	0.7	0.6	44.6	93.7
∠	0.0005		0.0005		0.01	

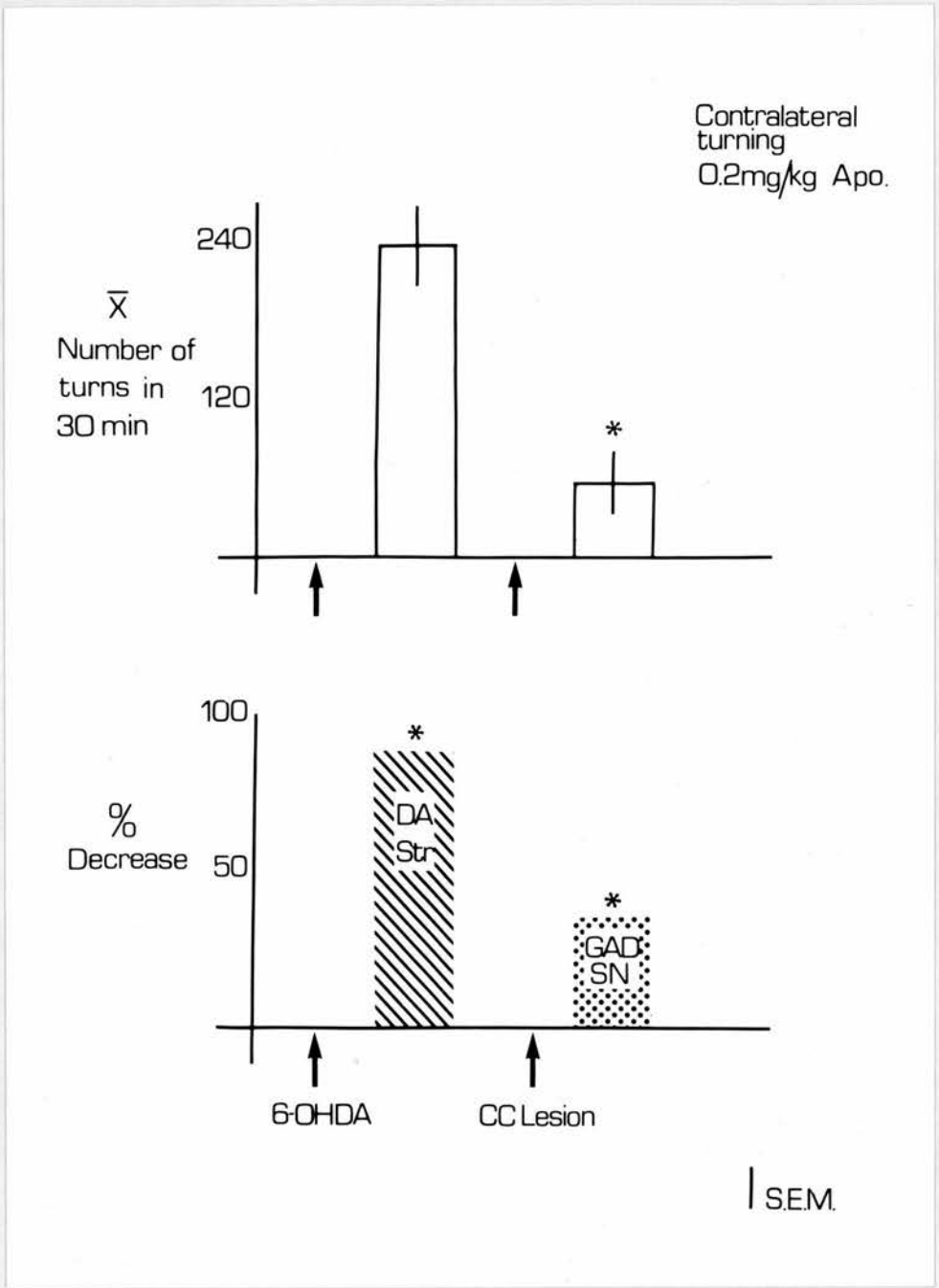


Fig. 37 Top Graph: Mean number of turns induced after apomorphine (0.2 mg/kg) in 6-OHDA lesioned rats, before and after a lesion in the striato-nigral pathway.

Lower Graph: Percentage decrease in dopamine in the striatum (Str) and in GAD activity in the substantia nigra (SN), compared to the unlesioned side.

*= difference statistically significant.

(Section D.4)

E. DISCUSSION.

The turning reported here after a lesion in the ventro-medial area of the crus cerebri, was towards the lesioned side independently of the drug, amphetamine or apomorphine. It can be proposed that these dopamine agonists act on the side where the striatal output to the substantia nigra is intact. Amphetamine induces a dose dependent rotation towards the substantia nigra lesioned side in a 6-OHDA injected animal, (44). It has been proposed that it acts presynaptically releasing dopamine on the intact side and this by an unknown effect in striatal cells causes turning behaviour. Apomorphine induces turning towards the unlesioned side, that is, opposite to the turning induced by amphetamine. It is believed that this dopamine agonist reaches postsynaptic receptors on both sides of the brain, but exerts a strongest effect on the denervated side, probably due to the development of postsynaptic supersensitivity, (285).

The mechanism by which turning is produced, surprisingly has remained a puzzle since turning behaviour was first studied, (5). Since rotation in a 6-OHDA lesioned animal is induced by dopamine agonists and blocked by dopamine antagonists, (e.g. haloperidol), it has been used as a technique for testing the efficacy of new drugs, (70, 17, 116), although it is not even clear why the animals display such behaviour.

The experiments presented here give a step towards the understanding of this motor behaviour. The lesion of the striato-nigral pathway considerably reduces the number of turns to apomorphine after a 6-OHDA lesion in the substantia nigra, (Section D.4). This points out that the striato-nigral pathway serves as an output involved in turning/

turning behaviour. A recent paper, (197) has reported similar findings in 6-OHDA lesioned animals. Lesions which effectively decreased the number of turns after apomorphine were localized in the striatum, globus pallidum or in the crus cerebri where the striato-nigral pathway runs. Therefore this also indicates that the lesions are affecting a part of the chain involved in turning behaviour.

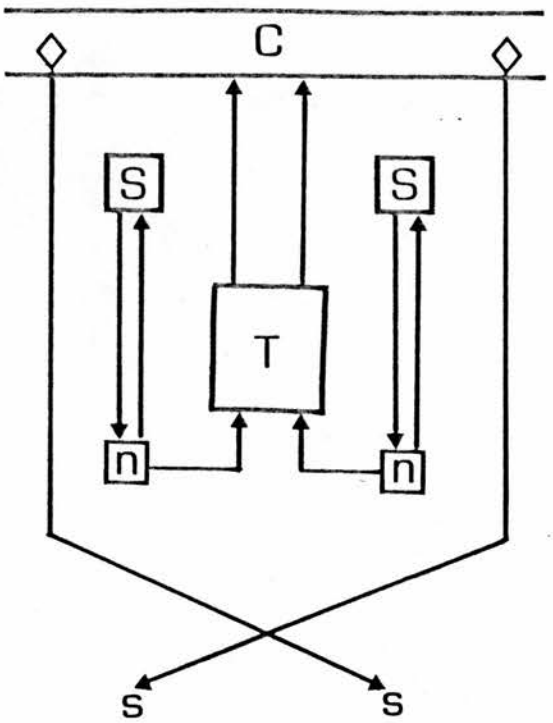
Apart from the motor alterations in humans in which the striatum and the substantia nigra participate, (e.g. Parkinson's Disease (147, 132) and Hungtington's Disease, (289, 30)), the ventrolateral thalectomy, has been used for the relief of tremor and rigidity in patients suffering from Parkinson's Disease, (250). After lesions in the ventrolateral nucleus of the thalamus, language and/or speech deficits are commonly observed in the patients, (250, 80). In monkeys it has been reported that a lesion in the ventro-lateral nucleus of the thalamus, produces moderate motor deficits during ballistically initiated flexion movements and dysmetria of the hind limbs contralateral to the lesion side, (243). These results point out that thalamic nuclei as well as the striatum and the substantia nigra, may be involved in a single circuit responsible for the motor alterations observed.

It has been reported that cells in the zona reticulata of the substantia nigra, which are inhibited from the caudate are also antidromically driven from the ventro-lateral and ventro-medial nucleus of the thalamus, (83). It has also been reported that stimulation in the substantia nigra pars reticulata produces an inhibition of cells in these thalamic nuclei. In 32% of the recorded neurons, the inhibition was observed in cells activated by cerebellar stimulation and antidromically driven after cortical stimulation, (84). This, together/

together with the anatomical reports indicating connections between the substantia nigra pars reticulata to the thalamus, (251, 56) and from there to the sensory-motor cortex, (63, 271, 82), provides evidence for the postulation of a circuit involved in the production of turning behaviour, (Fig. 38).

It can be suggested that manipulation of the concentration of dopamine in the striatum, will change the concentration of GABA in the substantia nigra pars reticulata which in turn will affect the nigro-thalamic input producing a change in excitability of pyramidal cells of the sensory-motor cortex, by means of the thalamo-cortical fibres. According to this, the motor cortex would be necessary for the turning behaviour to occur. In fact, it has been reported that cats and rats after the removal of their contralateral sensori-motor cortex, fail to show turning behaviour, either spontaneously or induced by drugs, previously observed after striatal lesion, (78, 79).

The reason why an animal with a lesion in the striatum or the crus cerebri shows turning behaviour towards the lesioned side after apomorphine or amphetamine could be because these drugs are increasing the activity of the striato-nigral pathway of the intact side, increasing the concentration of GABA in the substantia nigra and inducing turning opposite to the side having an increased amount of GABA. In agreement with this, it has been observed that the injection of GABA agonists, (e.g. muscimol and baclofen) into the substantia nigra induces rotation opposite to the injected side, (260, 228), and apomorphine increases the turnover of GABA in the substantia nigra,(235). Similarly, an increase in the amount of GABA in the substantia nigra by local injection of ethanolamine-O-sulphate an inhibitor of GABA glutamate transaminase, induces turning behaviour opposite to the injected side, (176, 294).



ig. 38 Schematic representation of a possible circuit involved in turning behaviour. C = sensory-motor cortex; S = striatum; n = substantia nigra; s = spinal cord; ◇ = pyramidal cells; T = thalamic nuclei.

Lesions in the substantia nigra pars reticulata induce turning ehaviour after apomorphine in the same direction as lesions in the triato-nigral pathway (Chapter III, Section D.4.2 and 87, 228). This n be explained if it is considered that both lesions are blocking the sponse induced by apomorphine on that side, so that only the cells of

e/

the thalamic nuclei on the intact side are being excited.

It is relevant to mention that as reported in Chapter II, Section D.3.1, rats with a lesion produced by kainic acid injection into the striatum, globus pallidum or ventro-lateral and reticular nuclei of the thalamus, also showed rotation towards the lesioned side. The fact that the rat with a thalamic lesion turned 192 times/30 min, strengthens the suggestion that this structure is involved in turning behaviour.

In order to test the circuit proposed it is necessary to study, among other things, the effects of the manipulation of GABA in the substantia nigra on the activity of the ventral nuclei of the thalamus as well as the activity of the pyramidal cells of the sensory-motor cortex.

Other motor alterations observed after a lesion of the substantia nigra, the striatum or the striato-nigral pathway, are probably affecting the same circuit. An example was given here with the changes in paw preference observed after contralateral lesions, to the preferred paw, in the striato-nigral pathway. Initially, the rats show a preference for a paw possibly because they have a natural assymetry in the concentration of dopamine in the striatum and in the concentration of GABA in the substantia nigra, which alters the excitability of cells in the ventro-lateral and ventro-medial nucleus of the thalamus and in turn the excitability of pyramidal cells of the sensory-motor cortex facilitating the use of one paw. This is certainly vague and imprecise, and research has to be done to establish if this is plausible. So far, it has been reported that normal rats also rotate consistently towards one side when given apomorphine, amphetamine or L-Dopa (151). The

contents/

contents of dopamine of the left and right striatum were found to differ by 10-15% in ordinary conditions and by 25% following a maximal dose of amphetamine, (20 mg/kg IP); the rats tended to turn opposite to the side with the highest level of dopamine, (122). Similarly, normal rats exhibit side preferences in a T-maze which is enhanced by amphetamine and coincide with the side of the brain containing the lowest concentration of dopamine in the striatum, that is the animal turned opposite to the substantia nigra containing the highest concentration of dopamine, (307). Changes in paw preference have already been reported. Stimulation of the striatum on the same side to the preferred paw causes a reversal of the side preference, (308) and the same effect is observed after a lesion of the striatum contralateral to the preferred paw, (257).

The adipsia and aphagia induced by lesions in the substantia nigra with 6-OHDA injection (283, 288) was not observed in animals with lesions of the striato-nigral pathway, although it was observed after the kainic acid injection into the striatum and globus pallidum, (Chapter II, Section D.3.1). It would be interesting to see if this alteration is also seen after bilateral lesions of the striato-nigral pathway, since unilateral lesions, even with 6-OHDA are less severe, (288). After unilateral lesions of the striato-nigral pathway difficulty in swallowing and frequent choking was observed when the animal was eating. This could be one of the possible reasons why the sham operated rat pressed a higher number of times than lesioned rats, in the post-operative test, (Section D.3., Fig. 36). A motor alteration and difficulty in swallowing may account for the adipsia and aphagia observed after 6-OHDA lesions, although a sensory impariment has also been/

been suggested, (286). Electrophysiological data from the substantia nigra in conscious monkeys emphazise their role in motor behaviour. Cells in the substantia nigra mainly increased their firing rate while the animal was moving the mouth, protruding the lips, moving the inferior maxilar or feeding, independently of the flavour of the injested food, (210). These observations also suggest that the adipsia and aphagia observed in animals after 6-OHDA injections into the substantia nigra may be due to a motor disability rather than an impairment in the sensory input or motivation.

The results presented in this Chapter illustrated the possibility of an alternative function for the striato-nigral pathway. Rather than serving for a feedback to the dopamine cells of the substantia nigra, it seems to provide a way of connecting the dopamine system with the motor areas.

E. SUMMARY

An electrolytic lesion in the striato-nigral pathway:

I - Induced turning behaviour towards the lesioned side after intraperitoneal injections of apomorphine or amphetamine. This response was dose-dependent.

II - Significantly reduced the number of turns induced after apomorphine in animals with 6-OHDA lesions in the substantia nigra.

III - Changed the preference of the paw used to press a lever in a Skinner box, providing the lesion was contralateral to the preferred paw.

GENERAL DISCUSSION.

The experiments presented here have the relevance of demonstrating with the use of a small and localized lesion in the striato-nigral pathway, that cells in the striatum do not control the metabolism of dopaminergic cell bodies in the substantia nigra.

As has already been discussed in Chapter I, there were doubts about the existence of this feedback system. Several laboratories working on the hypothesis from different angles arrived to the same conclusion: there must be alternative mechanisms of control of dopamine metabolism which will explain the effects of antipsychotic drugs, (256, 282, 120, 130). The alternative explanation of presynaptic control of dopamine release in the terminals of dopaminergic fibres within the striatum has been proposed, (Chapter I, Section 2, B.2.3). Also presynaptic control by means of gabaminergic and cholinergic neurons in the striatum was postulated, (Chapter I, Section 2.C.1).

Here, it was observed that a lesion in the striato-nigral pathway do not impair the ability of nigral cells to alter their turnover of dopamine after haloperidol or apomorphine, (Chapter II, Section D.4). In every case there was no difference in the concentration of dopamine or its metabolites HVA and DOPAC between control and lesioned rats.

These experiments were done at a time when release of dopamine was obtained from the substantia nigra, (73, 119) and when amphetamine was shown to inhibit dopaminergic cell firing, (2, 39) and induce release of dopamine from the substantia nigra, (217). This pointed out that there may be another alternative mechanism in the control of dopamine release involving dendro-dendritic interaction of axon collaterals from the dopaminergic cells which synapse on neighbouring cells. The decrease/

decrease in firing rate described in Chapter III, in control as well as lesioned animals, where no feedback was possible, clearly coincides with this hypothesis. The observation that dopaminergic cells when driven antidromically have a dissociation between the activity in the soma and dendrites (SD spike) and the spike of the internal segment, (IS), (138), gives evidence that there may be a cell to cell interaction inducing a hyperpolarization. Haloperidol can speed up cells by disinhibiting them from their lateral inhibition, blocking the action of dopamine and increasing the turnover of dopamine in the striatum. By which means the lateral inhibition takes place is a matter of argument, there is evidence of dendro-dendritic associations containg vesicles, (139) and this could be taken as the main evidence to postulate lateral inhibition produced by dendritic synapses. However a more recent report indicates that although there are dendro-dendritic appositions, there are no membrane especializations to indicate that synapses is occurring; membrane attachments, dense projections, vesicles or membrane thickenings were not observed between dendrites, (73). If this evidence is correct, then lateral inhibition must be produced by axon collaterals, but there is no direct evidence that axon collaterals of dopaminergic cells synapse in near by cells.

Recently it has been observed that while there is an increase in dopamine release in the striatum due to sensory stimulation (e.g. pressure in the skin, or light in an eye) or stimulation of the dentate or fastigial cerebellar nuclei, there is a decrease in dopamine release in the substantia nigra, (220, 216). It could be proposed that the increase in dopamine release in the striatum is due to activation of nigro-striatal fibres whereas the decrease in dopamine release observed in/

in the substantia nigra is due to the hyperpolarization induced by the cell to cell interaction in the substantia nigra. It would be interesting to record from dopaminergic cells after sensory or cerebellar stimulation in order to see if the cells are activated and then inhibited probably as a consequence of the proposed hyperpolarization.

Alternatives to the feedback system favouring the striatum or the substantia nigra as the site for control of dopamine metabolism, are not mutually exclusive, and since only one alternative does not explain all the evidence available for both structures, the regulation of dopamine metabolism and the effects of antipsychotic drugs, could either be mediated within each structure by any of the alternative mechanisms proposed or by an unknown biochemical process. The exclusive control of dopamine metabolism in the cell bodies cannot explain for instance the increase in TOH activity in the striatum after an interruption of the nigro-striatal fibres, or the increase in dopamine overflow after haloperidol administration in in-vitro studies, (Chapter I, Section 3.2). On the other hand, alternative explanations taking the striatum as the only site of control, cannot explain the decrease in firing rate observed after amphetamine in dopaminergic cells in the substantia nigra, after lesions in the striato-nigral pathway, (Chapter III).

There are other alternatives that must be considered before disregarding the possibility of a feedback mediated by a chain of neurons. For instance, lesions of the striato-nigral pathway do not interrupt all the routes along which the striatum might influence the substantia nigra. And afferents to the substantia nigra from other nuclei might be involved as well. Autoradiographic studies, (282,43) indicate/

indicate that the tail of the striatum sends fibres to the lateral portion of the substantia nigra pars reticulata and lateral areas of the substantia nigra pars compacta. However, since dopamine and GABA is found predominantly in the body of the striatum, (280, 146), whereas the tail contains mainly serotonin, (281), it is tempting to think that the pathway most likely involved for the postulated feedback should be the one from the body of the striatum interrupted by the ventro-medial lesions of the crus cerebri. In any case, the participation of this part of the striatum is also excluded, since lateral lesions of the crus cerebri interrupting this pathway, did not affect the metabolism of dopamine or the response of nigral cells to amphetamine or haloperidol, (Chapter II, Section D.4.1 and Chapter III, Section D.3.2).

The nucleus accumbens, the raphe nucleus, the habenula, the hypothalamus and the parietal, temporal and insular cortex seem to send afferents to the substantia nigra, (43, 272). Consistent anatomical results are obtained from the nucleus accumbens and the dorsal raphe, but so far there is no evidence of these nuclei participating in the control of dopamine metabolism in the striatum and substantia nigra. Stimulation of the medial raphe nucleus produces an inhibition in 56% of the cells tested, in the substantia nigra pars compacta, (89). Lesions of the medial raphe nucleus decrease the concentration of serotonin in the substantia nigra and increase the concentration of dopamine in the striatum, (89). This evidence has been taken as support to postulate that a mechanism which could affect dopamine metabolism of substantia nigral cells could be mediated by serotonin —containing fibres ending in the substantia nigra probably inhibiting —interneurons. This hypothesis proposes that a decrease in serotonin —in/

in the substantia nigra would produce a disinhibition of inhibitory interneurons which synapse on dopaminergic cells and as a result the firing rate of dopaminergic cells would decrease and the concentration of dopamine increase due to less dopamine utilization in the striatum, (89). It has been shown that dopamine agonists increase the release of serotonin in the rat brain measured by the levels of 5-hydroxyindolacetic acid (248). It would be interesting to study the effects of dopamine agonists and antagonists on dopamine turnover after a lesion in the medial raphe nucleus. If this nucleus is modulating dopamine metabolism in the way proposed by the feedback hypothesis, the alteration in dopamine turnover produced by these drugs should be greatly reduced after the lesion.

The lesions of the whole crus cerebri described in Chapter II (Table III) or hemisections between the striatum and the substantia nigra reported elsewhere (200), do not produce more than 70% decrease in the concentration of GABA or in GAD activity in the substantia nigra of the lesioned side. This means that the other 30% of the total concentration of GABA observed in the substantia nigra comes from another source behind the substantia nigra or is contained within the nucleus in GABA interneurons. As it has been just described, an inhibitory interneurone has been proposed to mediate the effects of serotonin on dopamine cells, (89), this interneurone could be a GABA containing one. However, other places like the fastigial and the dentate nucleus of the cerebellum have been suggested to be likely candidates to send afferents to the substantia nigra, as a result of biochemical evidence, but there is no direct anatomical evidence, (220). The role that this 30% of the total concentration of GABA plays in the control/

control of dopamine metabolism remains to be established.

Recently the existence of another nigro-striatal pathway apart from the dopamine containing one has been revived in an electrophysiological report. It has been found that cells from the substantia nigra pars compacta as well as from the pars reticulata show antidromic spikes to stimulation of the striatum. The fibres from the substantia nigra par compacta appear, as already reported, to be slowly conducting (0.3 - 1.0 m/sec) while the fibres from the pars reticulata have a faster conduction velocity (1.9 - 10 m/sec). The fibres leaving the substantia nigra pars reticulata seem to follow the same course as the dopamine containing ones, since antidromic activity could also be evoked stimulating the medial forebrain bundle, (85). The participation of these fibres in the metabolism of dopamine has not been studied. Although the cells giving rise to the fast nigro-striatal pathway lie within the pars reticulata, it is not know if the striato-nigral pathway establishes contact with them. Injections of colchicine, a drug that blocks axonal transport, into the globus pallidum and the striatum produces a build up of acetylcholinesterase within axons following a similar course to the one described for the dopamine containing pathway. This has been taken as evidence for a possible acetylcholine containing pathway from the substantia nigra to the striatum, (46). However there is always the possibility that the acetylcholinesterase observed is contained in the dopaminergic cells. It is also possible that the antidromic activity recorded in this second nigro-striatal pathway is the result of activation of nigro-cortical fibres travelling along the striatum. In fact, a nigro-cortical pathway has been described (15), which could be activated in its way

to/

to the cortex. The existence as well as the possible participation of this pathway in striatal function need to be confirmed.

It is interesting to note that a similar "feedback" regulation in the turnover of serotonin has been observed. Stimulation of serotoninergic receptors by lysergic acid diethylamide, (LDS), (7) and some hallucinogenic phenyl ethylamines, (10) decrease the turnover of serotonin in the brain and spinal cord of rats measured by the levels of serotonin after the inhibition of tryptophan hydroxylase. In contrast the administration of serotoninergic receptor blockers, (e.g. methysergide, methergoline) induce an increase of synthesis and turnover of serotonin in rats, (266). A feedback loop formed by a chain of neurons as in the dopaminergic system has been postulated in order to explain the results, (7). However, no anatomical evidence exists of a feedback loop of the kind postulated for the dopamine system in nuclei containing serotonin like the raphe nuclei. It is very interesting that as in the case of in vitro studies of dopamine release, the "feedback" mechanisms are still operating in brain slices for the release of labelled serotonin induced by electrical field stimulation, (35). Thus LSD (receptor agonist) and methiothepin (antagonist), added to the incubating medium, exert opposite effects on the efflux of serotonin. These results suggest that other mechanism than a feedback loop is involved in the regulation of transmitter release in the serotoninergic as well as in the dopaminergic neurons.

According to the feedback hypothesis a decrease in the concentration of GABA should impair the ability of nigral cells to respond to dopamine agonists and antagonists, since this response is supposed to be mediated by GABA fibres ending in dopaminergic cell bodies in the substantia/

substantia nigra. Apart from the evidence already discussed in Chapter I, Section A.2 and D.1.1 and Chapter II, Section E.3 about the GABA-dopamine relation, turning behaviour has been used as an estimate of this relation. An increase in the content of GABA in the substantia nigra after a local injection of ethanolamine-O-sulphate, an inhibitor of GABA glutamate transminase, induces turning behaviour contralateral to the injected side, and stereotyped sniffing and biting which are intensified by systemic amphetamine administration. This has been taken as evidence to suggest that an increase in GABA levels in the substantia nigra, results in increased functional activity of the dopaminergic containing nigro-striatal pathway (176). However, these same results can be explained with the circuit described in Chapter IV, Section E, as the result of an increased activity of cells in the substantia nigra pars reticulata, sending afferents to thalamic nuclei, and from there to the sensory-motor cortex. Evidence in favour of this suggestion and against a GABA-dopamine relation, has also been obtained using turning behaviour as an estimate. Unilateral injections of the GABA agonist muscimol into substantia $nigr_a$, induced turning contralateral to the injected side even after pretreatment with dopamine antagonist haloperidol, (294). It would be interesting to investigate the effects of 6-OHDA in the substantia nigra on the turning induced by an increase in GABA transmission in the substantia nigra. According to what has been proposed a lesion of dopaminergic cells should not affect the turning induced by increased GABA or GABA agonists into substantia nigra.

The results presented in Chapter III, open the possibility of a different function for the striato-nigral pathway conveying information out/

out of the striatum as part of a circuit involved in the expression of motor behaviour. There are many reports involving the striatum and the substantia nigra in learning. For instance, it has been found that lesions of the striatum impair the recall of a learned task or the ability to acquire a new one, (86, 188, 240). Injections of L-Dopa into the striatum improve tasks which require the animal to stand still, such as go no-go situations, (172). On the other hand electrical stimulation of the substantia nigra pars compacta during the acquisition of a passive avoidance task impairs the retention of the correct response, (258), and bilateral lesions of the substantia nigra with 6-OHDA, appart from inducing severe akinesia, impair the ability of rats to learn an underwater maze, (246, 116). As well as the proposed involvement in learning, stimulation or lesion of these structures produces alteration in motor performance. For instance, stimulation of the striatum induces movement of the head and turning behaviour (269), similarly nigral lesioned animals appart form their akinetic state, have a correlation between the number of dopamine cells left intact, and the ability of the animal to swim when forced in an underwater maze, (245). Since it has been reported that unilateral lesions, do not produce the significant results observed after bilateral lesions, it would be very interesting if animals with bilateral lesions of the striato-nigral pathway have similar deficits. Since the striato-nigral pathway is probably mediating the output of the striatum to thalamus and cortical areas, (Chapter Iv, Section E.) it would be expected to affect behaviour in a similar way than bilateral lesions of the striatum. Also, if the effect of substantia nigral lesions is due to the lack of dopamine in the striatum induced by the lesion, bilateral lesions/

lesions of the striato-nigral pathway should impair behaviour in a similar way.

Over the past few years progressive evidence of the synthesis, storage, release and catabolism of dopamine has helped in the understanding of the metabolism of synaptic transmitter and the mode of action of several drugs. However, neurochemical, pharmacological and electrophysiological evidence of the dopamine containing cells is not at all complete. The anatomical and biochemical techniques developed over the last few years have contributed enormously to the knowledge of these neurons and their interelations, and more contributions appear every day. Nevertheless probably all these processes are much more complicated than the simple logic that we are trying to apply. If one agrees with Einstein that "God does not play dice with the universe," the apparent incomplete and chaotic state of the understanding of neuronal function and its interelations, must become clearer as knowledge develops. But let's also bear in mind as Professor Hawkins once pointed out, "God not only plays dice, He sometimes throws the dice where they cannot be found"!

BIBLIOGRAPHY

1 Aghajanian, G.K. and Bunney,B. (1973) Central DA neurons: neurophysiological identification and responses to drugs. In Fronteirs in Catecholamine Research, 643-648.

2 Aghajanian, G.K. and Bunney, B.S. (1977) Dopamine "autoreceptors": Pharmacological characterization by microintophoretic single cell recording studies. Naunyn-Schmiedebergs Arch. Pharmacol., 297.

3 Alderman, J.L. and Shellinberger, M.K. (1974) GABA in the rat brain: Re-evaluation of sampling procedures and the postmortem increase. J. Neurochem. 22, 932-940.

4 Anden, N.E., Dahlstrom, A., Fuxe, K. and Larson, K. (1965) Further evidence for the presence of nigro-neostriatal dopamine neurons in the rat. Am. J. Anatomy, 116, 329-339.

5 Anden, N.E., Dahlstrom, A., Fuxe, R. and Larson, K. (1966) Functional role of nigro-neostriatal DA neurons. Acta Pharmacol. (Kbh) 24, 263-274.

6 Anden, N.E., Rubenson, A., Fuxe, K. and Kokfelt, T. (1967) Evidence for DA receptor stimulation by apomorphine. J. Pharma. Pharmacol. 19, 627-628.

7 Anden, N.E., Corrodi, H., Fuxe, K. and Kokfelt, T. (1968) Evidence for a central 5-HT receptor stimulation by lysergic acid diethylamide. Br. J. Pharmacol., 34, 1-7.

8 Anden, N.E., Corrodi, H., Fuxe, K. and Ungersted, U. (1971) Importance of nervous impulse flow for the neuroleptic induced increase in amine turnover in central dopamine neurons. European J. Pharmac. 15, 193-199.

9 Anden, N.E. and Svensson, T.H. (1973) Release of DA from central N.A. nerves, after treatment with reserpine plus amphetamine. J. Neurol. Transmission, 34, 23-30.

10 Anden, N.E., Corrodi, H., Fuxe, K. and Meek, J. (1974) Hallucinogenic phenyleyhylamine: interactions with serotonin turnover and receptors. Eur. J. Pharmacol., 25, 176-184.

11 Anden, N.E. and Grabowska, M. (1975) Synthesis and disappearance of central NA and DA: Regulation via nerve impulses and receptor activity. Chem. Tools in Catechol. Res. Vol. II, 143-149

12 Amer, M.S. and Kreighbaum, W.E. (1975) Cyclic nucleotide phosphodiesterase: properties, activators, inhibitors, structure activity relationships and possible role in drug development. H. Pharma. Sc. 64, 1-37.

13 Arbuthnott, G.W. and Ungersted, U. (1975) Turning behaviour induced by electrical stimulation of the nigro-striatal system of the rat. Exp. Neurol. 47 (1) 162-172.

14 Arbuthnott, G.W., Mitchell, M.J., Tulloch, I.F. and Wright, A.K. (1976) Efferent pathways from lateral hypothalamic neurons. J. Physiol 263, 131-132 P.

15 Avedano, C., Reinoso-Suarez, F., Llamas, A. (1976) Projections of gyrus sigmoideus from the substantia nigra revelead by HRP retrograde transport technique. Neuroscience Lett. 2, 61-65.

16 Balcon, G.J., Lenox, R.H. and Meyerhoff, J.L. (1975) Regional GABA levels in rat brain determined after microwave fixation. J. Neurochem., 24, 609-613.

17 Baldessarini, B.J., Walter, K.G., and Borgman, R.G. (1975) Esters of apomorphine and N, N-dimethyldopamine as agonists of DA receptors in the rat brain in vivo. Neuropharmacol. 14, 725-731.

18 Bartholini, G., Stadler, H., Eadea-Ciria, M. and Lloyds, K.G. (1974) The effects of antipsychotic drugs on the release of neurotransmitters in various brain areas. In: Sedvall, G., Uvnas, B. and Zotterman, Y. (eds.) Antipsychotic drugs: pharmacodynamics and pharmacokinetics Pergamon Press, 105-116.

19 Bartholini, G. and Stadler, H. (1975) Cholinergic and GABA-ergic influence on the Dopamine release in extrapyramidal centres. In Algrem et al (1975) (eds.) Chem. Tools in Catechol. Res. Vol. II 235-241.

20 Bartholini, G., Stadler, H., Gadea-Ciria, M. and Lloyde, K.G. (1977) Interaction of dopaminergic and cholinergic neurons in the extrapyramidal and limbic systems. In Costa, E. and Gessa, G.L. (eds) Adv. in Bioch. Psycholpharmacol., Vol. 16, 391-395.

21 Beuer, J. (1909) Die Substantia nigra Soemmeringii; Eine vergleichendl-anatomische Studie nebst einenr Beitrag zur Kenntris des dunkeln Pigments der Nervenzellen. Arb. a.d. Neurol. Inst. a.d. Wien. Univ. Band 17; 435-512.

22 Bedard, P., Larochelle, L. Parent, A. and Poirier, L.J. (1969) The nigrostriatal pathway: a correlative study based on neuroanatomical and neurochemical criteria in the cat and the monkey. Exp. Neurol, 25, 365-377.

23 Bernardi, G., Marciani, G., Morocutti, C. and Giacomini, P. (1976) The action of picrotoxin and bicuculline on rat caudate neurons inhibited by GABA. Brain Res. 102, 379-384.

24 Bernardini, G., Flrois, V., Marciani, M.G., Morocutti, C. and Stanzione, P. (1976) The action of Ach. and L-glutamic acid on rat caudate neurons. Brain Res. 114, 134-138.

25 Bernardini, G., Marciani, M.G., Morocutti, C. and Giacomini, P. (1975) The action of GABA on rat CN recorded intracellularly. B. Res. 92, 511-515.

26 Bernstein, A.L. (1964) A handbook of statistic solutions for the behavioural sciences. Holt, Rinehart and Winston, Inc.; 32-35.

27 Besson, M.J., Cheramy, A. and Glowinsky, (1971) Effects of some psychotropic drugs on dopamine synthesis in the rat striatum. J. Pharmacol. Exp. Ther., 196-205.

28 Bevan, P. Bradshaw, C.M. and Szabadi, E. (1975) Effects of desipramine on neuronal responses to DA, NA, 5-HT and Ach in the caudate nucleus of the rat B.J. Pharmacol. 54, 285-293.

29 Bevan, P., Bradshaw, C.M. and Szabadi, E. (1975) Effects of iprindole on responses of single cortical and caudate neurons to monoamines and Ach. Br. J. Pharmac., 55, 17-25.

30 Bird, E.D. and Iversen, L.L. (1974) Huntington's chorea. Post-mortem measurement of GAD, CAT and DA in Basal Ganglia. Brain, 97, 457-472.

31 Bjorklund, A. and Lindvall, O. (1975) DA in dendrites of SN neurons: suggestions for a role in dendritic terminals. Brain Res. 83, 3, 531-537.

32 Blaustein, M.P., Johnson, E.M. Jr. and Needleman, P (1972) Calcium-dependent norepinephrine release from presynaptic nerve endings in rats. Proc. Nat. Acad. Sci. U.S.A., Vol. 69, 8 2232-2240.

33 Bloom, F.E., Costa, E., Salmoiraghi, G.C. (1965) Anaesthesia and the responsiveness of individual neurons of the caudate nucleus of the cat to Ach., NE and DA administered by microelectrophoresis. J. Pharmac. and Exp. Therap. 150, 2, 244-252.

34 Bloom, F.E. (1975) Physiological assessment of pre- and post-synaptic receptors. In Usdin, E. and Bunney, W.E. (eds) Pre- and postsynaptic receptors. Marcel Dekker Inc., 73.

35 Bourgoin, S., Artaud, F., Enjalbert, A., Henry, F., Glowinski, J. and Hamon, M. (1977) Acute changes in central serotonin metabolism induced by the blockade or stimulation of serotoninergic receptors during ontogensis in the rat. J. Pharmac. Exp. Ther. 202, 519-531.

36 Brodal, A. (1969) Neurological Anatomy, 2nd Ed., Oxford University Press.

37 Brown, N. (1973) In Electronics for neurobiologists, 402-405.

38 Buchwald, N.A., Hull, C.D., Levine, M.S. (1975) Neurophysiological and anatomical inter-relationships of the basal ganglian in 133, 187-204.

39 Bunney, B.S., Aghajanian, G.K., and Roth, R.H. (1973) Comparison of effects of L-Dopa, Amphetamine and Apomorphine on firing rate of rat dopaminergic neurons. Nature, new biol. 245, 123-125.

40 Bunney, B.S. and Aghajanian, G.K. (1973) Electrophysiological effects of amphetamine on DA neurons. In frontiers of catecholamine Research, 957-962.

41 Bunney, B.S., Walters, J.R., Roth, R.H. and Aghajanian, G. (1973) Dopaminergic neurons: effect of antipsychotic drugs and amphetamine on single cell activity. J. of Pharmacol. and Exp. Ther. 185, 3, 560-571.

42 Bunney, B.S. and Aghajanian, G.K. (1976) d-Amph-induced inhibition of central dopaminergic neurons: Mediation by a striato-nigral feedback pathway. Science, 192, 391-393.

43 Bunney, B.S. and Aghajanian, G. (1976) The precise localizations of nigral afferents in the rat, as determined by a retrograde tracing technique, Brain Res. 117, 423-435.

44 Bunney, B.S. and Aghajanian, G.K. (1976) The effect of antipsychotic drugs on the firing of Dopaminergic neurons: A reappraisal. In Sedvall, G., Uvnas, B.J., Zotterman, Y. Antipsychotic drugs: Pharmacodynamics and pharmacokinetics Pergamon Press.

45 Burt, D.R., Enna, S.J., Crese, I. and Snyder, S.H. (1975) DA receptor binding in the Striatum of mammalian brain. Proc. Nat. Acad. Sc. 72, 11, 4655-4659.

46 Butcher, L.L. (1977) Nature and mechanisms of cholinergic-monoaminergic interactions in the brain. Life Sci. 21, 1207-1226.

47 Carenzi, A., Cheney, D., Costa, E., Guidotti and Racagni, G. (1975) Action of opiates, antipsychotics, amphetamine and apomorphine on DA receptors in rat striatum: In vivo changes of 3' - 5' cyclic AMP content and Ach turnover rate. Neuropharmacol. 14, 927-939.

48 Carlsson, A. and Lindquist, M. (1963) Effect of Chlorpromazine or Haloperidol on formation of 3-Methoxytyramine and normet-anephine in mouse brain. Acta. pharmacol. et Toxicol. 20, 140-144.

49 Carlsson, A., Kehr, W., Lindquist, M., Magnusson, T., Atack, C.V., (1972) Regulation of monoamine metabolism in the CNS. Pharmacol. Rev. 24, 371-398.

50 Carlsson, A. (1975) Drugs acting through DA release. Pharmac. Therap. B, 1, 3, 401-405.

51 Carlsson, A. (1975) Dopaminergic autoreceptors. In Almgren, O., Carlsson, A. and Engel, J. (eds). Chem. Tools in Catecholamine Res. Vol. II, 219-225.

52 Carlsson, A. (1975) Receptor-mediated control of dopamine metabolism. In Usdin, E. and Bunney, W. (eds.) Pre- and post-synaptic receptors. Marcel Dekker, Inc. 49-66.

53 Carlsson, A. (1977) Dopaminergic Autoreceptors: Back-ground and implications. In Costa, E. and Gess, G.L. (eds) Advances Biochem. Pharmacol. Vol. 16, 439-441.

54 Carman, J.B., Cowan, W.M., Powell, T.P.S. and Webster, K.E. (1965) A bilateral corticostriate projection. J. Neurol. Neurolog. Psychiat., 28, 71-77.

55 Carpenter, M. and Peter, P. (1972) Nigrostriatal and nigrothalamic fibres in the Rhesus monkey. J. Comp. Neurol., 144, 93-116.

56 Carpenter, M.B., Makano, K. and Kim, R. (1976) Nigrothalamic projections in the monkey demonstrated by autoradiographic technics. J. Comp. Neurol. 165, 401-416.

57 Carter, D.A. and Fibiger, H.C. (1977) Ascending projections of presumed DA-containing neurons in the ventral tegmentum of the rat as demonstrated by Horseradish Peroxidase. Neuroscience, 2, 569-576.

58 Cattabeni, F., Maggi, A., Angelis, L., De, Bruno, F. and Racagni, G. (1977) A simple approach to study the effect of haloperidol on GABA functional activity in the nigro-striatal system. J. Neurochemistry, 28, 1407-1408.

59 Chang, M.M. and Leeman, S.E. (1970) Isolation of a sialogogic peptide from borine hypothalamic tissue and its characterization as substance P. J. Biol. Chem., 245, 18, 4784-4790.

60 Cheney, D.L., Zsilla, G. and Costa, E. (1977) Acetylocholine turnover rate in N. Accumbens, N. Caudatus, Globus Pallidus and Substantia Nigra. Action of cataleptogenic and Non-cataleptogenic Antipsychotics. In. Costa, E. and Gessa, G.L. (eds.) Advances in Biochemical Psychopharmacol. Vol. 16, 179-185.

61 Cherany, A., Besson, M.J., Gauchy, C., Javoy, F., Agid, Y., Guyenet, P., Glowinski, J. (1974) In vivo techniques for the analysis of biochemical effects of antipsychotic drugs. In Sedvall, G., Uvnas, B. and Zotterman, Y. (eds) Antipsychotic drugs, Pharmacodynamics and Pharmacokinetics. Pergamon Press, 161-173.

62 Chiara, G. Di., Porceddu, M.L., Fratta, W., Gessa, G.L. (1977) Postsynaptic receptors are not essential for dopamine feedback regulation. Nature, 267, 270-271.

63 Chow, K.L., and Pribram, K.H. (1956) Cortical projection of the thalamic ventro-lateral nuclear group in monkeys. J. Comp. Neurol., 104, 57-76.

64 Connor, J.D. (1968) Caudate unit responses to nigral stimuli: evidence for a possible nigro-neostriatal pathway. Science 160, 899-900.

65 Connor, J.D. (1975) Electrophysiology of the nigro-caudate DA pathway. Pharmac. Therap. B., 1, 3, 357-370.

66 Cools, A.R. (1973) Chemical and electrical stimulation of the CN in freely moving cats. The role of DA. Brain Res. 58 437-451.

67 Coombs, J.S., Eccles, J.C. and Fatt, P. (1955) The electrical properties of the motoneurone membrane J. Physiol, 130, 291-325.

68 Coombs, J.S., Curtis, D.R. and Eccles, J.C. (1957) The interpretation of spike potentials of motorneurons. J. Physiol.

69 Corrodi, H., Fuxe, K. and Hokfelt, T. (1967) The effect of some psychoactive drugs on central monoamine neurons. Eur. J. Pharmacol 1: 363-368.

70 Corrodi, H. Farnebo, L.O., Fuxe, K., Hamberger, B. and Ungersted, U. (1972) ET 495 and brain catecholamine mechanisms: evidence for stimulation of DA receptors.

71 Coyle, J. and Snyder, S.H. (1969) Catecholamine uptake by synaptosomes in homogenates of rat brain: stereospecificity in different areas. J. Pharmacol. Exp. Therap., 170, 2, 221-231.

72 Cowan, W.M., Gottliebs, D.I. Hendrickson, A.E., Price, J.L. and Woolsey, T.A. (1972) The autoradiographic demonstration of axonal connections in the central nervous system. Brain Res. 27, 21-51.

73 Cuello, A.C., Iversen, L.L. (1977) Interactions of DA with other neurotransmitters in the rat SN: a possible functional rolse of dendritic, D.A: - Dendritic release of DA - In Garattini, S., Prijol, J.F. and Samanin, R. (eds.) International Symposium on interactions among pulative neurotransmitters in the brain. Raven Press.

74 Cuello, A.C., Jesse[illegible] T.M., Kanazawa, I. and Iversen, L.L. (1977) Substance P: local[illegible]tion in synaptic vesicles in central nervous system. J. of Neurochem. 27, 747-751.

75 Crow, T.J. (1972) A map of the rat mescencephalon for electrical self-stimulation, Brain Res. 36, 265-273.

76 Crossman, A.R. Walter, R.J. and Woodruff, G.N. (1973) Picrotoxin antagonism of GABA inhibitory responses and synaptic inhibition in the rat SN. British J. Pharmacol. 49, 696-698.

77 Crosby, E. (1962) Correlative anatomy of the nervous system. MacMillan.

78 Dankova, J., Boucher, R., Poirier, Z.J., (1975) Role of the strio-pallidal system and motor cortex in induced circus movements in rats and cats. Exp. Neurol. 47, 135-149.

79 Dankova, J., Boucher, R., Poirier, L.J. (1977) Effects of 1694 and other DA agents on circling behaviour. E.J. Pharmacol. 42, 113-121.

80 Darley, T., Brown, J. and Swenson, W. (1975) Language changes after neurosurgery for Parkinsonism, Brain & Language, 2, 65-69.

81 Davies, J. and Dray, A. (1976) Substance P in the substantia nigra Brain Res. 107, 623-627.

82 Deniau, J., Lackner, D. and Feger, J. (1973) Etude chez le chat avec au sans cortex moteur du control des cellules due nogau ventral lateral du thalamus par la substance noire. J. Physiol. Paris, 66, 481-493.

83 Daniau, J. Feger, J., Le Guyder, C. (1976) Striatal evoked inhibition of identified nigro thalamic neurons. Brain Res. 104, 152-156.

84 Deniau, J.M., Lackner, D., Feger, J. (1978) Effect of SN stimulation on identified neurons in the VL-VA Thalamic complex; comparison between intact and chronically decorticated cats. Brain Res. 145, 27-35.

85 Deniau, J.M., Hammond, C., Riszk, A. and Geger, J. (1978) Electrophysiological properties of identified output neurons of the rat Substantia Nigra (pars compacta and pars reticulata): Evidences for the existence of branched neurons. Exp. Brain Res. 32, 409-422.

86 Divac, I., Rosvold, E. and Szwarcbart, M.K. (1967) Behavioural effects of selective ablation of the caudate nucleus. J. Comp. Physiol. Psychol., 63, 2, 184-190

87 Dray, A., Fowler, L.J., Oakley, N.R. (1975) Proceedings: Comparisons of circling behaviour following unilateral inhibitions of GABA - transaminase on discrete electrolytic lesioning in the rat SN. Br. J. Pharmacol. 55, 2, 288 P.

88 Dray, A., Gonye, T.J., and Oakley, N.R. (1976) Caudate stimulation and SN activity in the rat. J. Physiol. 259, 3, 825-849.

89 Dray, A., Conye, T.J., Oakley, N.R. and Tanner, T. (1976) Evidence for the existence of a raphe projection to the substantia nigra in rats. Brain Res., 113, 45-57.

90 Drummond, R.S. and Phillips, A.T. (1974) L-glutamic acid decarboxylase in non-neural tissues of the mouse. J. Neurochem., 23, 1307-1213.

91 Eccles, J.E. (1964) The physiology of synapses. Spring-Verlag, 228.

92 Enna, S.J., Bennett, J.P., Burt, D.R., Creese, I. and Snyder, S.H. (1976) Stereospecificity of interaction of neuroleptic drugs with neuro transmitters and correlation with chemical potency. Nature, 263, 338-340.

93 Ernst, M.A. (1967) Mode of action of apomorphine and dexamphetamine on gnawing compulsion in rats. Psychopharmacologia, 10, 316-323.

94 Eulervon, S., Pernow, B. (1977) Substance P. Raven Press.

95 Fahn, S. and Cote, L.J. (1968) Regional distribution of GABA in brain of the rhesus monkey. Brain Res., 15, 209-213.

96 Falck, B. (1962) Observations on the possibilities for the cellular localization of monoamines with a fluorescence method. Acta Physiol. Scand., Suppl. 197.

97 Farnebo, L.O. and Hamberger, B. (1971) Drug induced changes in the release of 3H-monamines from field stimulated rat brain slices. Acta. Physiol. Scand. Suppl. 371, 35-44.

98 Farnebo, L.O., Hamberger, B. (1973) Catecholamine release and receptors in brain slices. In frontiers of Catecholamine Research. Pargamon Press. 589-593.

99 Faul, R. and Carman, J.B. (1968) Ascending projections of the substantia nigra in the rat. J. Comp. Neurol. 132, 73-92.

100 Faul, R.L.M., and Laverty, R. (1969) Changes in DA levels in the corpus striatum following lesions in the SN. Exp. Neurol. 23, 332-340;

101 Feltz, P. and MacKenzie, J.S. (1969) Properties of caudate unitary responses to repetitive stimulation. Brain Res. 13, 612-616.

102 Feltz, P. (1969) Dopamine, aminoacids and caudate unitary responses to nigral stimulation. J. Physiol. 205, 8-9p.

103 Feltz, P. (1971) GABA and a Caudato-Nigral inhibition. Can. J. Physiol. Pharmacol. 49, 1113-1115.

104 Feltz, P. (1971) Sensitivity to haloperidol of caudate neurons excited by nigral stimulation. E.J. of Pharmacol. 14, 360-364.

105 Feltz, P. and Albe-Fessard, D. (1972) A study of an ascending nigro-caudate pathway. EEG and Clin. Neurophysiol, 33 179-193.

106 Feltz, P., De Champlain, J. (1973) The postsynaptic effect of amphetamine on striatal DA - sensitive neurons. In Frontiers of Catecholamine Research 951-956.

107 Feger, J. Ohye, C., Gallowin, F. and Albe-Fessard, D. (1975) Stereotaxic technique for stimulation and recording in non-anaesthetized monkeys: application to the determination of connections between CN and SN. Advances in Neurology, Vol. 10, 35-45.

108 Feger, J. and Ohye, C. (1975) The unitary activity of the SN following stimulation of the striatum in the awake monkey. Brain Res. 89, 155-159.

109 Fibiger, H. and Phillips, A. (1976) Retrograde amnesia after electrical stimulation of the SN: mediation by the DA nigro-neostriatal bundle. Brain Res. 116, 23-33.

110 Fifkova, E. and Syka, J. (1964) Relationships between cortical and striatal spreading depression in rat. Exp. Neurol., 9, 355-366.

111 Fonnum, F., Grofova, I., Rinvik, E., Storm-Mathisen, J. and Walberg, F. (1974) Origin and distribution of GAD in substantia nigra of the cat. Brain Res. 71, 77-92.

112 Fourtes, M.G.F., Frank, K. and Becker, M.C. (1957) Steps in the production of motoneuron spikes, J. Gen. Physiol. 40, 735-752.

113 Frigyesi, T.L., and Purpura, D.P. (1967) Electrophysiological analysis of reciprocal caudate-nigral relations. Brain Res. 6, 440-456.

114 Frigyesi, T.L. and Szabo, J. (1975) Caudate evoked synaptic activities in nigral neurons. Exp. Neurol. 99, 123-139.

115 Fuxe, K. and Ungersted, U. (1976) Antiparkinsonian drugs and Dopaminergic neostriatal metchanisms: studies in rats with unilateral 6-OHDA induced degeneration of the nigro-striatal DA pathway and quantitative recording of rotational behaviour. Pharmac. Ther. B. 2, 41-47.

116 Gaddy, J., Neill, D. (1977) Differential behavioural changes following intrastrital application of 6-OHDA. Brain Res. 119, 439-446.

117 Gale, K., Guidotti, A., and Costa, E. (1977) DA-sensitive adenylate cyclase: Location in SN. Science 195, 503-504.

118 Gauchy, C., Bioulac, B., Cheramy, A., Besson, M.J., Glowinski, J. and Vincini, J.D. (1974) Estimation of chronic DA release from the CN of the macaca mulatta. Brain Res. 77, 257-268.

119 Geffen, L., Jessell, T., Wells, C., Ivessen, L. (1976) Release of DA from dendrites in rat SN. Nature 260, 258-260.

120 Giorguieff, M.F., Le Floch, M.L., Glowinski, J. and Besson, M.J. (1977) Involvement of Cholinergic presynaptic receptors of nicotinic and muscarinic types in the control of the spontaneous release of DA from striatal DA terminals in the rat. J. Pharmacol. Exp. Ther. 200, 535-544.

121 Giorguieff, M.E., Kennel, M.L., Glowinski, J. and Besson, M. (1978) Stimulation of DA release by GABA in rat striatal slices. Brain Res. 139, 115-130.

122 Glick, D.S., Crane, A.M., Jerussi, T.P., Fleisher, L.D., Green, J.P., (1975) Functional and neurochemical correlates of potentiation of striatal asymmetry by callosal section. Nature, 254, 616-617.

123 Glowinski, J., Agid, Y., Besson, M.J., Cheramy, A., Gauchy, C. and Jaroy, F. (1973) Regulation of DA synthesis in the nigro neostriatal system. In Frontiers of Catecholamine Research. Pergamon Press. 605-611.

124 Grafstein, B. (1977) Axonal transport: the intra cellular traffic of the neuron. In Brookhart, J.M. and Mountcastle, V.B. Handbook of Physiology: The nervous system, 1, 691-717.

125 Greengard, P. Mathason, J.A., Kebabian, J.N., (1973) DA, octopamine and serotonin-sensitive adeylate cyclases; possible receptors in aminergic neurotransmission. In Frontiers of CA Research, Pergamon Press. 377-382.

126 Greengard, P. (1974) Biochemical characterization of the DA receptor in the mamalian caudate nucleus J. Psychiatric Res. 11, 87-90.

127 Greengard, P. (1974) The action of antipsychotic drugs on DA-stimulated adenylate cyclase activity. In Sedvall, G., Uvnas, B. and Zotterman, Y. (eds.) Antipsychotic drugs: pharmacodynamics and pharmacokinetics. Pergamon Press, 271-283.

128 Grofova, I. (1975) The identification of striatal and pallidal neurons projecting to SN. An experimental study by means of retrograde axonal transport of horseradish peroxidase. Brain Res. 91, 286-291.

129 Groves, P., Wilson, C., Young, S.J., Rebec, G. (1975) Self Inhibition by dopaminergic neurons: An alternative to the "neuronal feedback loop" hypothesis for the mode of action of certain psychotropic drugs. Science 190, 522-529.

130 Groves, P.M., Young, S.J., and Wilson, C.J. (1976) Self inhibition of Dopaminergic neurons: disruption by(±) -alpha-methyl-para-tyrosine pretreatment of anterior diencephalic lesions. Neuropharmacol. 15, 755-762.

131 Goldstein, M., Backstrom, T., Ohi, Y. and Frankel, R. (1970) The effects of Ca ions on the C^{14} - catecholamine biosynthesis from C^{14}-tyrosine in slices from the striatum of rats. Life Sci. 9, 919.

132 Goldstein, M. and Anagnoste, B. (1976) Monkeys with nigrostriatal lesions: effects of monoaminergic drugs. Pharmac. Ther. B., 2, 97-103.

133 Goldstein, M., Bronaugh, R.L., Lew, J.Y., Ohashi, Y., Drummon, G.S. and Fuxe, K. (1977) Effect of DA agents and cAmp on TOH activity. In Costa, E. and Gessa, G.L. (eds) Advances in Biochemical Psychopharmacol. Vol. 16, 447-453.

134 Goswell, M.J. and Sedgwick, E.M. (1971) Inhibition in the Substantia Nigra following stimulation of the caudate nucleus. J. Physiol. 218, 84-85 P.

135 Guidotti, A. and Gale, K. (1977) Participation of GABA receptors in the short-term activation of striatal tyrosine-3-Monooxygenase elicited by neuroleptics. In Costa, E. and Gessa, G.L. (eds) Advances in Biochemical Pharmacology, Vol. 16, 455-464.

136 Gulley, R.L. and Wood, R.L. (1971) The fine structure of the neurons in the rat substantia nigra. Tissue and Cell, 3, 675-690.

137 Guyenet, P.G., Agid, Y., Javoy, F., Beaujouan, J.C. Rossier, J. and Glowinski, J. (1975) Effects of DA receptor agonists and antagonists on the activity of the neo-striatal cholinergic system. Brain Res. 84, 227-244.

138 Guyenet, P.G. and Aghajanian, G.K. (1978) Antidronic identification of dopaminergic and other output neurons of the rat SN. Brain Res. 150, 69-84.

139 Hajdu, F., Hassler, R., Bak, I.J. (1973) Electron microscopic study of the substantia nigra and the strio-nigral projection in the rat. Z. Zellforsch 146, 207-221.

140 Hattori, T., Fibiger, H. and McGeer, P. (1975) Demonstration of a Pallido-nigral projection innervating DA neurons. J. Comp. Neurol. 162, 487-504.

141 Hattori, T., Singh, U.K., McGeer, E.E. and McGeer, P.L. (1976) Immunohistological localization of choline acetyl transferase containing neurostriatal neurons and their relationship with dopaminergic synapses. Brain Res. 102, 164-173.

142 Hays, W.L. (1973) Statistics for the social sciences. Holt, Rinehart and Winston, Inc., 424-427.

143 Hedrera, J.C. (1971) Separate demonstration of dopaminergic and non-dopaminergic projections in the rat. Anat. Rec. 169, 338.

144 Hellon, R.F. (1971) The marking of the electrode tip positions in nervous tissue. J. Physiology, 214, 12P.

145 Herz, A. and Sreglansbergen, W. (1966) Synaptic excitation in the corpus striatum inhibited by microelectrophoretically administered DA. Experientia 22, 839-840.

146 Hong, J.S., Yang, H-Y.T. and Costa, E. (1977) Projections of SP containing neurons from neostriatum to substantia nigra. Brain Res., 122, 541-544.

147 Hornykiewicz, U. (1966) Dopamine, (3-Hydroxytyramine) and brain function. Pharmacological Reviews, 18, 2, 925-964.

148 Hull, C.D., Bernardi, G. and Buchwald, N.A. (1970) Intracellular responses of caudate neurons to brain stem stimulation. Brain Res. 22, 163-179.

149 Inversen, L.L., Horn, A.S. and Miller, R.J. (1974) Structure-activity relationships for interactions of agonist and antagonist drugs with DA-stimulated adenylate cyclase of rat brain - a model of CNS DA receptors? In Sedvall, G., Uvnas, B., and Zotterman Y. (eds) Antipsychotic drugs: pharmacodynamics and pharmacokinetics. Pergamon Press, 285-303.

150 Janssen, A.J. (1967) The pharmacology of haloperidol. Int. J. Neuropsych. (August), 10-18.

151 Jerussi, T.P. and Glick, S.D. (1975) Apomorphine induced rotation in normal rats and interaction with unilateral caudate lesions. Psychopharmagologia, 40, 329-334.

152 Jessell, T., Irevsen, L.L. and Kanazawa, I. (1976) Release and metabolism of SP in rat hypothalamus. Nature 264, 81-83.

153 Jessell, T.M. (1978) SP release from the rat SN. Brain Res. 151, 469-478.

154 Johnson, T.N. and Clements, C.D. (1959) An experimental study of the fiber connections between the putamen, globus pallidus, ventral thalamus and midbrain tegmentum in cat. J. Comp. Neurol. 113, 84-101.

155 Juorio, A.V., Sharman, D.F. and Trajkov, T. (1966) The effect of drugs on the HVA content of the corpus striatum of some rodents. Br. J. Pharmacol. 26, 385-392.

156 Juraska, J., Wilson, C. and Groves, P. (1977) The Substantia Nigra of the rat. A golgi study. J. Comp. Neurol. 172, 585-600.

157 Kanazawa, I., Marshall, G.R. and Kelly, J.S. (1976) Afferents to the rat SN studied with HRP with special reference to fibres from the subthalamic nucleus. Nrain Res. 115, 185-491.

158 Kanazawa, I. and Jessell, T. (1976) Postmortem changes and regional distribution of Substance P in the rat and mouse nervous system. Brain Res, 117, 362-367.

159 Kanazawa, I. and Jessell, T. (1976) Post-mortem changes and regional distribution of SP in the rat and mouse nervous system. Brain Res. 117, 362-367.

160 Kanazawa, I. Emson, P., Cuello, C. (1977) Evidence for the existence of SP-containing fibres in strio-nigral and pallido-nigral pathways in rat brain. Brain Res. 119, 447-453.

161 Kebabian, J.W., Petzold, G.L. and Greengard, P. (1972) DA-sensitive adenylate cyclase in caudate nucleus of the rat brain, and its similarity to the "DA-receptor". Proc. Nat. Acad. Sa., (U.S.A.), 69, 2145.

162 Kebabian, J.W. and Greengard, P. (1971) Dopamine sensitive adenyl cyclase: Possible role in synaptic transmission. Science, 174, 1346.

163 Kebabian, J. and Saavedra, J. (1976) DA-sensitive adenylate cyclase occurs in a region of SN containing DA dendrites. Science, 193, 683-685.

164 Kehr, W., Carlsson, A., Lindgvist, M., Magnusson, T. and Atack,
165 C.V. (1972) Evidence for a receptor mediated feedback control of striatal TOH activity. J. Pharm. Pharmacol. 24, 744-747.

166 Keller, H.H., Bartholini, G., Pieri, L. and Pletscher, A. (1972) Effects of spreading depression on the turnover of cerebral dopamine, Eur. J. Pharmacol. 20, 287-290.

167 Kemp, J.M., and Powell, T.P.S. (1971) The synaptic organisation of the caudate nucleus. Phil. Trans. R. Soc. Lond. B. 262, 403-412.

168 Kemp, J.M. and Powell, T.P.S. (1971) The connections of the striatum and globus pallidus: synthesis and speculation. Phil. Trans. R. Soc. Lon. B. 262, 441-452.

169 Kim, J.S., Bak, I.J., Hassler, R. and Okada, Y. (1971) Role of GABA in the extrapyramidal motor system. 2. Some evidence for the existence of a type of GABA -rich strio-nigral neurons. Exp. Brain Res., 14, 95-104.

170 Kitai, S.T., Wagner, A., Precht, W. and Ohno, T. (1975) Nigro-caudate and caudato-nigral relationship: an electrophysiological study. Brain Res. 85, 44-48.

171 Kitai, S.T., Kocsis, J.D., Preston, R.J., Sugimoni, M. (1976) Monosynaptic inputs to caudate neurons identified by intracellular injection of HRP. Brain Res. 109, 601-606.

172 Kitsikis, A. (1975) L-Dopac-induced improvement of conditioned response inh. In Buchwald, N.A. and Brazier, M. (eds.) Brain Mechanisms in mental retardation. Academic Press, 219-224.

173 Kluver, H. and Barrera, E. (1953) A method for the combined statining of cells and fibres in the NS. J. Neuropath. exp. Neurol. 12, 400-403.

174 Konig, J.F.R. and Klippel, R.A. (1963) The rat brain: A stereotaxic Atlas of the forebrain and lower parts of the brain stem. Williams and Wilkins, Baltimore, Md.

175 Koniski, S. and Otsuka, M. (1974) Excitatory action of hypothalamic substance P on spinal motorneurons of newborn rats. Nature, 252, 734-735.

176 Koob, G., Fiacco. M. and Iversen, S. (1976) The behavioural effects of EOS-induced changes in the SN GABA levels. Br. J. Pharmacol. 58, 454P.

177 Korf, J., Aghajanian, G.K., and Roth (1973) Alterations in turnover and endogenous levels of NE in cerebral cortex following stimulation and acute axotomy of cerebral NE pathways. E.J. Pharmacol. 23, 276-282.

178 Kristersson, K. (1970) Transport of fluorescent protein tracer in peripheral nerves. Acta neuropath. (Berl.) 16, 293-300.

179 Kristersson, K., Olsson, Y. and Sjostrand, J. (1971) Axonal uptake and retrograde transport of exogenous proteins in the hypoglossal nerve. Brain Res. 32, 399-406.

180 Krnjevic, K. and Morris, M.E. (1974) An excitatory action of Substance P on cuneate neurons. Cand. K. Physiol. Pharmacol. 52, 736.

181 Laduron, P.M., Janssen, P.F.M. and Leysen, J.E. (1978) Spiperone: a ligand of choice for neuroleptic drugs. Biochem. Pharmacol. 27, 317-321.

182 Lasek, R.J. (1970) Protein transport in neurons. Int. Res. Neurobiol. 13, 289-324.

183 Lashley, K.S. (1950) In search of the engram. In physiological mechanisms in animal behaviour. Soc. Exp. Biol. Symp. 4, 454-482

184 Laursen, A.M. (1963) Corpus striatum. Acta physiol. Scand. 59, 211.

185 La Vail, J. and La Vail, M. (1972) Retrograde axonal transport in the central nervous system. Science, 176, 1416-1417.

186 Leysen, J., Niemegeers, C.J., Tollenagre, J. and Laduson, P. (1978) Serotoninergic component of neuroleptic receptors. Nature, 272, 168-171.

187 Leysen, J.E., Gommeren, W. and Laduron, P.M. (1978) Spiperone: a ligand of choice for neuroleptic receptors: Kinetics and characteristics of in vitro bindings. Biochem. Pharmacol. 27, 307-316.

188 Livesey, P.J. and Rankine-Wilson, J. (1975) Delayed alternation learning under electrical (blocking) stimulation of the caudate nucleus in the cat. J. Comp. Physiol. Psychol. 88, 1, 342-354.

189 Lloyd, K.G., Shibuya, M., Davidson, L. and Hornykiewicz, O. (1977) Chronic Neuroleptic Therapy: Tolerance and GABA systems. In Costa, E. and Gessa, G.L. (eds.) Advances in Biochemical Phsychopharmacol. Vol. 16, 409-415.

190 Loren, I., Bjorklund, A., Falk, B. and Lindvall, O. (1976) An improved histo fluorescence procedure for freeze dried paraffin-embedded tissue based on combined formaldehyde-glyoxylic acid perfusion with high magnesium content and acid pH. Histochemistry, 49, 177-192.

191 Lorell, R.A. and Elliott, K.A.C. (1963) GABA and factor I content of brain. J. Neuroch. 10, 479-488.

192 Lorenberg, W., Ames, M. and Lerner, P. (1977) Regulation of tyrosine hydroxylase activity. In Costa, E. and Gessa, G.L. "Nonstriatal Dopaminergic Neurons". Advances in Biochemical Psychopharmacology. Vol. 16, 461-464.

193 Lowrey, O.H., Rosebrought, N.J., Farr, A.L. and Randall, R.S. (1951) Protein measurement with the folin phenol reagent. J. Biol. Chem., 193, 265.

194 Mao, C.C., Cheney, D.L., Marco, E., Revuelta, A. and Costa, (1977) Turnover times of GABA and Ach in NC., N Acc, GP and SN: effects of repeated administration of haloperidol. Brain Res. 132, 2, 375-379.

195 Marco, L.A., Copack, P. and Edelson, A. (1973) Intrinsic connections of caudate neurons. Locally intracellular responses. Exp. Neurol. 40, 3, 683-698.

196 Marsden, C.A. and Kerkut, G.A. (1969). The cellular localization of monoamines in invertebrates using the Edwards-Pearse tissue dryer. In Kerkut, G.A. (ed.) Experiments in physiology and biochemistry, Vol. II, 327-360.

197 Marshall, J.F. and Ungersted, U. (1977) Striatal efferent fibres play a role in maintaining rotational behaviour in the rat. Science, 198, 62-64.

198 McAfee, D.A., Schorderet, M. and Greengard, P. (1971) Adenosine 3', 5'-monophorphate in nervous tissue: Increase associated with synaptic transmission, Science, 171, 1156.

199 McAfee, D.A. and Greengard, P. (1972) Adenosine 3', 5'-monophosphate: electrophysiological evidence for a role in synaptic transmission. Science, 178, 310.

200 McGeer, P.L., Fibiger, H.C., Hattori, T., Singh, U.K., McGeer, E.G. and Maler, L. (1974) Biochemical neuroanatomy of the basal ganglia. In Myers, R.D. and Drucker, Colin, R.R. (eds) Advances in behavioural biology, vol. 10, 27-48.

201 McGeer, P.L., McGeer, E.G., Singh, U.K. and Chase, W.H. (1974) Choline acetyltransferase localization in the CNS by immunohistochemistry. Brain Res. 81, 373-379.

202 McKenzie, G.M. and Szerb, J.C. (1968) The effect of dihychoxphenylalanine, pheniprazine and dextroamphetamine on the in vivo release of DA from the caudate nucleus. J. Pharmacol. Exp. Ther. 162, 2, 302-308.

203 McLennan, H. and York, D.H. (1967) The action of dopamine on neurons of the caudate nucleus. J. of Physiol. 189, 393-403.

204 McNair, J.L., Sutur, J. and Tsubokawa, T. (1972) Depression of cell firing in the SN by caudate nucleus stimulation. Exp. Neurol. 37, 395-411.

205 Mersah, P. and Deadwyler, S. (1974) The caudate nucleus of the rat cell types and the demonstration of a commissural system. J. Anat. 117, 281-293.

206 Mishra, R.K., Gardner, E.L., Latzman, R. and Makman, M.H. (1974) Enhancement of DA stimulated adenylate cyclase activity in the rat caudate after lesions in SN: evidence for DA deveration Proc. Nat. Acad. Sc. 71, 10, 3883-3887.

207 Moleman, P., Bruinvels, J. and Westerink, B.H.C. (1977) Rapid post mortem changes in 3, 4-dihydroxy phenylacetic acid (DOPAC) a dopamine metabolite, in rat striatum. J. Neurochem. 29, 175-177.

208 Moore, L. (1963) Toxicity and CA releasing action of d- and L-amphetamine in isolated and aggregated mice. J. Pharmacol. Exp. Ther. 142.

209 Moore, R.Y., Bhatnagar, R.K. and Heller, A. (1971) Anatomical and chemical studies of a nigro-neostriatal projection in the cat. Brain Res. 30, 119-135.

210 Mora, F., Mogenson, C.J., and Rolls, E.T., (1977) Activity of neurons in the region of the SN during feeding in the monkey. Brain Res. 133, 267-276.

211 Mathason, J.A. (1977) Cyclic Nucleotides and nervous system function. Physiol. Rec. 57, 2, 156-256.

212 Nauta, W.J.H. and Mehler, W.R. (1961) Some efferent connections of the lentiform nucleus in monkey and cat. Anat. Rec. 139, 260.

213 Nauta, H.J., Pritz, M.B. and Lasek, R.J. (1974) Afferents to the rat caudatoputamen studied with horseradish peroxidase. An evaluation of a retrograde neuronatomical research method. Brain Res. 67, 219-238.

214 Nicolaou, N. (1978) Study of some influences on the nigro-striatal dopaminergic pathway of the rat brain with particular reference to the influence of 5-HT. Thesis presented for the degree of Doctor in Philosphy, Fac. Medicine, Edinburgh University.

215 Nieoullon, A., Cheramy, A., Glowinski, J. (1977) Interdependence of the nigro striatal DA system on the two sides of the brain in the cat. Science, 198, 416-418.

216 Nieoullon, A., Cheramy, A. and Glowisky (1977) Nigral and striatal DA release under sensory stimuli. Nature, 269, 340-342.

217 Nieoullon, A., Cheramy, A., Glowinski, J. (1977) Release of DA in vivo from act SN. Nature, 266, 375-377.

218 Nieoullon, A., Cheramy, A. and Glowinski, J. (1977) An adaptation of the push-pull cannula method to study the in vivo release of (3H) DA synthesized from (3H) tyrosine in the cat caudate nuclus: effect of various physical and pharmacological treatments. J. Neurochemistry, 28, 819-828.

219 Nieoullon, A., Cheramy, A. and Glowinski, J. (1978) Release of DA evoked by electrical stimulation of the motor and visual areas of the cerbral cortex in both CN and in the SN in the cat. Brain Res. 145, 69-83.

220 Nieoullon, A., Cheramy, A. and Glowinski, J. (1978) Release of DA in both caudate nuclei and both substantia nigra in response to unilateral stimulation of cerebellar nuclei in the cat. Brain Res. 148, 143-152.

221 Nyback, H. and Sedvall, G. (1969) Regional accumulation of catecholamines formed from tyrosine 14C in rat brain. Effect of chloropromazine Eur. J. Pharmac. 5, 245-252.

222 Nyback, H. (1971) Regional disappearance of catecholamines formed from 14C-Tyrosine in rat brain. Effect of synthesis inhibitors and chloropromazine Acta. Pharmacol. Tex. 30, 372-384.

223 Nyback, H.U., Murin, C.L., Sedvall, G.C., Roth, R.H. (1974) Pharmacological and electrical stimulation of DA turnover in the rat brain. In Sedvall, G., Uvnas, B. and Zotterman Y. (eds) Antipsychotic drugs: pharmacodynamics and pharmacokinetics. Pergamon Press, 175-182.

224 Nyback, H. (1975) On the relation between transmitter turnover and impulse flow in brain catecholamine neurons. Chem. Tools in Catecholamines Research, Vol. II, 127-134.

225 Nyback, H., Sedvall, G. (1976) Effects of neuroleptics on striatal DA synthetised from 14C Tyrosine. Pharmac. Ther. B.2, 49-63.

226 Okada, Y., Nitsch-Hassler, C., Kim, J.S., Bak, I.J. and Hassler, R. (1971) Role of GABA in the extrapyramidal motor system. 1. Regional distribution of GABA in rabbit, rat, guinea pig and baboon, CNS. Exp. Brain Res. 13, 514-518.

227 Okada, Y. and Shimada, C. (1975) Distribution of GABA and GAD in the guinea pig hippocampus. Brain Res. 98, 202-206.

228 Olianas, M.C., Montis, G.M de., Mulas, G. and Tagliamonte, A. (1978) The striatal dopaminergic function is mediated by the inhibition of a nigral, non-dopaminergic neuronal system via a strio-nigral gabaergic pathway. Eur. J. Pharmacol. 49, 233-241.

229 Olney, J.W., Rhee, V., Ho. O.L. (1974) Kainic acid a powerful neurotoxic analogue of gluatamate. Brain Res. 77, 507-512.

230 Olpe, H.R., Koella, W.P., Wolf, P. and Haas, H.L. (1977) The action of Baclofen on neurons of the SN and ventral tegmental area. Brain Res. B, 4, 577-580.

231 Palkorits, M., Brownstein, M., Saaredra, J.M. and Axebrod, J. (1974) Norepinephrine and dopamine content of hypothalamic nuclei of the rat. Brain Res. 77, 137-149.

232 Parent, A. and Pairier, J.L. (1969) The medial forebrain bundle and ascending monoaminergic pathways in the cat. Can. J. Physiol Pharmacol. 47, 781-785.

233 Pearson, J.D.M. and Sharman, D.F. (1975) A method for the estimation of GABA using gas liquid chromatography and election capture detection. J. Neurochemistry, 24, 1225-1228.

234 Pearson, J.D.M. and Sharman, D.F. (1975) The estimation of 3,4-Dihydroxyphenylacetic acid, homovallinic acid and homo-isovanillic acid in nervous tissue by gas-liquid, chromatography and election capture collection. Br. J. Pharmacol. 53, 143-148.

235 Perez de la Mora, M.J., Fuxe, R., Hokfelt, T. and Ljungvahl, A. (1977) Evidence for a nerve impulse-dependent GABA accumulation in the SN after treatment with gamma-glutamyldydrazide. Neurosci. Letters, 5, 1-2, 75.

236 Phillipson, G.T., Emson, P.C., Hound, A.S. and Jessell, T. (1977) Evidence concerning the anatomical location of the DA stimulated adenylate ayclase in the SN. Brain Res. 136, 45-48.

237 Phillips, J.W. and Limacher, J.J. (1974) Substance P excitation of cortical Betz cells. Brain Res. 69, 458-163.

238 Pletscher, A. (1974) Interference of antipsychotic drugs with cerebral monamine turnover. In Sedvall, G., Uvnas, B. and Zotterman, Y. (eds) Antipsychotic drugs: pharmacodynamics and pharmacokinetics. Pergamon Press, 207-215.

239 Powell, D., Leeman, S., Tregear, G.W., Niall, H.D. and Potts, J.T. (1973) Radioommunoassay, for SP. Nature, 241, 252-254.

240 Prado-Alcala, R., Grinberg, J., Arditti, L., Garcia-Munoz, M., Prieto, G. and Brust-Carmura, H. (1975) Learning deficits produced by chronic and reversible lesions of the capus striatum in rats. Physiol. Beh., 15, 283-287.

241 Precht, W. and Yoshida, M. (1971) Blockage of caudate evoked inhibition of neurons in the SN by picrotoxin. Brain Res. 32, 229-233.

242 Ranck, James, Jr. (1975) Which elements are excited in electrical stimulation of mammalian, CNS: A review. Brain Res. 98, 417-440.

243 Ranish, N. and Soechting, J. (1976) Studies on the control of some motor tasks. Effects of thalamic and red nuclei lesions. Brain Res. 102, 339-345.

244 Ranje, C. and Ungersted, U. (1977) Discriminative and motor performance in rats after interference with DA neurotransmission with spiroperidol. Eur. J. Pharmacol. 43, 39-46.

245 Ranje, C. and Ungersted, U. (1977) High correlations between number of DA cells, DA levels and motor performance. Brain Res. 134, 83-93.

246 Ranje, C. and Ungersted, U. (1977) Lack of adquisition in DA derevated animals tested in an underwater y-maze. Brain Res. 134, 95-110.

247 Ranson, S.W., Ranson, S.W. and Ranson, M. (1941) Fibre connections of copus striatum as seen in marchi preparations. Arch. Neurol. Psychiat, 46, 230-249.

248 Reid, W.D.(1970) Turnover rate of brain 5-HT increased by d-amphetamine. Br. J. Pharmacol., 40, 483-491.

249 Richardson, T.L., Miller, J.J. and McLennan, H. (1977) Mechanisms of excitation and inhibition in the nigro-striatal system. Brain Res. 127, 219-234.

250 Riklan, M. and Cooper, I. (1975) Psychometric studies of verbal functions following thalamic lesions in humans. Brain and Language, 2, 45-64.

251 Rinvik (1975) Demonstration of nigrothalamic connections in the cat by retrograde axon transport of horseradish peroxidase. Brain Res. 90, 313-318.

252 Roberts, E. (1974) GAMA-Aminobutynic acid and nervous system function. - A perspective Biochem. Pharmacol. 23, 2637-2649.

253 Roos, B.E. (1969) Decrease in HVA as evidence for DA receptor stimulation by apomorphine in the neostriatum of the rat. J. Pharm. Pharmacol. 21, 264-264.

254 Roth, R.H., Walters, J.R., Aghajanian, G.K. (1973) Effect of impulse flow on the release and synthesis of DA in the rat striatum. Frontiers in catecholamine research, 567-574.

255 Roth, R.H., Salzman, P.M. and Morgenroth, H.V. (1974) Noradrenergic neurons, Allosteric activation of hippocampal TOH by stimulation of the locus coeruleus. Biochem. Pharmacol. 23, 2779-2784.

256 Roth, R.H., Walters, J.R., Murrin, L.C., and Morgenroth, U.H. (1975) Dopamine neurons. Role of impulse flow and pre-synaptic receptors in the regulation of tyrosine hydroxyclase. In Usdin, E. and Bunney, W.E. (eds) Pre and Postsynaptic Receptors, Marcel Dekker, Inc., 5-48.

257 Rothman, A. and Glick, S. (1976) Differential effects of unilateral and bilateral caudate lesions on side reference and passive avoidance behaviour in rats. Brain Res. 118, 361-369.

258 Routtenberg, A. and Holzman, N. (1973) Memory disruption by electrical stimulation of the SN pars compacta. Science, 181, 83-86.

259 Rundles, W. and Papey, J. (1937) Connections between the striatum and the substantia nigra in a human brain. Arch. Neurol. Psych. 38, 1, 550-562.

260 Scheel-Kruger, J., Arnt, J. and Magelund, G. (1977) Behavioural stimulation induced by muscinol and other GABA agonists injected into the SN. Neuroscience Letters, 4, 351-356.

261 Schwartz, S. (1970) Effects of DA, mescaline and substantia nigra stimulation on caudate neurons. C.I.N.D., VII, Congr. Neuro Psychopharmacol., II, 392. Excerpta Medica.

262 Schwarcz, R. and Coyle, J. (1977) Striatal lesions with kainic acid: neurochemical characteristics. Brain Res. 127, 235-249.

263 Schwarcz, R., Creese, I., Coyle, J.T. and Snyder, S.H. (1978) Dopamine receptors localized on cerebral cortical afferents to rat corpus striatum. Nature, 271, 766-768.

264 Schwyn, R.C. and Fox, C.A. (1974) The primate SN: A golgi and electron microscopic study. Vogt., Hirnforschung, Bd. 15, Heft, 1, 97-126.

265 Serman, P., Lee. T., Chow-Wong, M. and Wong, K (1976) Antipsychotic drugs doses and neuroleptic/DA receptors. Nature, 261, 717-718.

266 Sofia, R.D. and Vassar, H.B. (1975) The effect or ergotamine and methysergide on serotonin metabolism in the rat brain. Arch. Int. Pharmacodynam. Ther. 216, 40-50.

267 Spano, P.F., Trabucchi, M. and Di Chiara, G. (1977) Localization of nigral DA sensitive adenylate cyclase on neurons originating from the corpus striatum. Science, 196, 1343-1345.

268 Spehlmann, R. (1975) The effects of Ach. and DA on the CN depleted of biogenic amines. Brain 98, 219-230.

269 Stevens, J.R., Kim, C. and McLean, P.D. (1961) Stimulation of caudate nucleus. Arch. Neurol. (Chic) 4, 47-54.

270 Stone, T.W. (1973) Cortical pyramidal tract interneuron and their sensitivity to L-glutamine Acid J. Physiol. 233, 211-225.

271 Strick, P.L. (1970) Cortical projections of the feline thalamic nucleus ventralis lateralis. Brain Res. 20, 130-134.

272 Swanson, L.W. and Cowan, N.M. (1975) A note on the connections and developments of the nucleus accumbens. Brain Res. 92, 324-330.

273 Szabo, J. (1962) Topical distribution of the striatal efferents in the monkey. Exp. Neurol. 5, 21-36.

274 Szabo, J. (1967) The Efferent projections of the putamen in the monkey. Exp. Neurol. 19, 463-476.

275 Szabo, J. (1968) Efferent projections from the body of the caudate nucleus. Anat. Rec. 160, 437.

276 Szabo, J. (1969) Projections from the tail of the caudate nuclei Anat. Rec. 163, 272.

277 Szabo, J. (1970) Projections from the body of the caudate nucleus in the rhesus monkey. Experimental Neurology, 27, 1-15.

278 Toppaz, M.L., Brownstein, M.J. and Palkorits, M. (1976) Distribution of GAD in discrete brain nuclei. Brain Res. 108, 371-379.

279 Toppaz, M., Brownstein, M.J. and Kopin, I. (1977) Glutamate Decarboxylase (GAD and GABA in discrete nuclei of hypothalamus and substantia nigra. Brain Res. 125, 109-121.

280 Tassin, J.P., Cheramy, A., Blanc, G. and Glowinski, J. (1976) Topographical distribution of dopamine innervation and dopamine receptors in the rat brain. I. Miscroestimation of H-Da uptake and dopamine content in microdiscs. Brain Res. 107, 291-301.

281 Ternaux, J.P., Henry, F., Bourgoin, S., Adrien, J., Glowinski, J. and Hamon, M. (1977) The topographical distribution of serotoninergic terminals in the neostriatum of the rat and caudate nucleus of the cat. Brain Res., 121, 311-326.

282 Tulloch, I.F., Arbuthnott, G.W., and Wright, A.K. (1978) Topographical organisation of the striato-nigral pathway revealed by antedograde and retrograde neuroanatomical tracing techniques. Journal of Anatomy (in press).

283 Ungersted, U. (1971) Use of intracerebral injections of 6-OHDA as a tool for morphological and functional studies on central catecholamine neurons. In Malmforss, T. and Thoenern, H. (eds) 6-OHDA and catecholamine neurons. North-Holland, 315-332.

284 Ungersted, U. (1971) Histochemical studies on the effect of intra cerebral and intraventricular injections of 6-Hychoxydopamine on monoamine neurons in the rat brain. In Malmforss, T. and Thoenen, H. (eds) 6-Hychoxydopamine and catecholamine neurons North-Holland, II, 101-128.

285 Ungersted, U. (1971) Stereotaxic mapping of the monoamine pathways in the rat brain. Acta. Physiol. Scand. 367, 1-122.

286 Ungersted, U. (1971) Adipsia and aphagia after 6-OHDA induced degeneration of the nigro-striatal DA system. Acta. Physiol. Sc. 367, 95-122.

287 Ungersted, U. (1976) 6-OHDA induced degeneration of the nigro-striatal DA pathway: the turning synchome. Pharm. Ther. B. 2, 37-40.

288 Ungersted, U., Ljunberg, T. and Ranje, C. (1977) DA neurotransmission and the control of behaviour. In Cools, A.R. et al (eds) Psychobiology of the Striatum. North-Holland 85-97.

289 Urquhart, U., Perry, T.L., Hansen, S. and Kennedy, J. (1975) GABA contents and GAD activity in brain of Huntington's chorea patients and control subjects. J. Neurochemistry, 24, 1071-1075.

290 Verhaart, W.J. (1950) Fibre analysis of the basal ganglia. J. Comp. Neurol. 93, 425-440.

291 Vicg d'Azyr, F. (1786) Traite de'anatomie et de physiologie, 72-96 Paris.

292 Voigtlander, von, P.F., Moore, K.E. (1973) Involvement of nigro-striatal neurons in the in vivo release of DA by amphetamine amantadine and tyramine. J. of Pharmac. and Exp. Ther. 184, 3, 542-552.

293 Voneida, T.J. (1960) An experimental study of the course and destination of fibres arising in the head of the caudate nucleus in the cat and monkey. J. Comp. Neurol. 115, 75-87.

294 Waddingtton, J.L. (1978) Rotational behaviour and striatal dopamine metabolism following unilateral activation of nigral GABA mechanisms: GABA like modulation of dopaminergic and non-dopaminergic neurons in rat substantia nigra. Br. J. Pharmacol. 63, 2, 384P.

295 Walter, R.J., Kemp, J.A., Yajima, H., Kitagawa, K. and Woodruff, G.N., (1976) The action of substance P on mesencephalic reticular and substantia nigral neurons of the rat. Experientia, 32/2, 214-215.

296 Walters, J.R. and Roth R.H. (1972) Effect of gamma-hydroxybutyrate on DA and DA metabolites in the rat striatum. Biochem. Pharmacol. 21, 2111-2121.

297 Walters, J.R. and Roth, R.H. (1976) DA neurons: An in vivo system for measuring drug interactions with presynaptic receptors. Naunyn-Schmiedeberg's Archives of Pharmacol. 296, 5-14.

298 Weiss, P.A. (1969) Neuronal dynamics and neuroplasmic (axonal) flow. Symp. Int. Soc. Calf. Biol. 8, 3-34.

299 Willies, W. Jr., and Grossman, R.E. (1973) Medical Neurobiology The CV Mosby Co.

300 Wilson, C.J., Young, S.J. and Groves, P.M. (1977) Statistical properties of neuronal spike trains in the substantia nigra cell types and their interaction. Brain Res. 136, 243-260.

301 Winer, J.A. (1977) A review of the status of the Horseradish Peroxidase Method in Neuroanatomy. Biobehavioural Reviews, Vol. I 45-54.

302 York, D.H. (1970) Possible DA Pathway from SN to putamen. Brain Res. 20, 233-249.

303 York, D.H. (1972) DA receptor blockade - A central action of chloropromazine on striatal neurons. Brain Res. 37, 91-99.

304 York, D.H. (1976) Electrophysiology of the nigro-putamen DA pathway. Pharmac. Ther. B. 2, 1-7.

305 Yoshida, M. and Precht, W. (1971) Monosynaptic inhibition of neurons of the SN by caudate-nigral fibres. Brain Res. 32, 225-228.

306 Yoshida, M., Rabin, A. and Anderson, M. (1972) Monosynaptic inhibition of pallidal neurons by axon collaterals of caudate nigral fibres Exp. Brain Res. 15, 333-347.

307 Zimmenberg, B., Glick, S.D. and Jerussi, T.P. (1974) Neurochemical correlate of a spatial preference in rats. Science, 185, 623-625.

308 Zimmerberg, Betty and Glick, S. (1975) Changes in side preference during unilateral electrical stimulation of the CN in rats.

APPENDIX I

PUBLISHED PAPERS

STATEMENT IN TERMS OF Ph.D. REGULATION 2.4.11 and 6.7. OF THE POSTGRADUATE STUDY PROGRAM OF THE UNIVERSITY OF EDINBURGH

Some of the results described in this thesis have been presented as follows:-

Arbuthnott, G.W., Garcia-Munoz, M., Nicolaou, N., Tulloch, I.F., and Wright, A. (1976) Is the striato-nigral pathway responsible for "feedback" control of dopamine release? Br. J. Pharmacol. 58, 272 P.

Garcia-Munoz, N., Nicolaou, N.M., Tulloch, I.F., Wright, A. and Arbuthnott, G.W. (1977) Feedback loop or output pathway in striato-nigral fibres? Nature, 265, 5592, 363-365.

Wright, A., Arbuthnott, G.W., Tulloch, I.F., Garcia-Munoz, M. and Nicolaou, N. (1977) Are the striatonigral fibres the feedback pathway? In Cools, A.R., Lohman, A.H.M. and van der Berken, J.H.L. (ed.) Psychobiology of the Striatum, North-Holland Press, 31-50.

Arbuthnott, G.W., Tulloch, I.F., Wright, A., Nicolaou, N.M. and Garcia-Munoz, M. (1977) The functional significance of the striatonigral pathway in the rat brain. XXVII International Congress of Physiological Sciences, Paris:; Proceedings.

Tulloch, I.F., Arbuthnott, G.W., Wright, A.K., Garcia-Munoz, M. and Nicolaou, N.M. (1978) The striatonigral fibres and the feedback control of dopamine metabolism. Psychol. Med., 8, 471-482.

Garcia-Munoz, M., Arbuthnott, G.W. (1978) The role of the striatonigral pathway in the control of the substantia nigra. In: Catecholamines: Basic and Clinical Frontiers, Pergamon Press. (In press)